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Mercury Concentration of White Muscle in Relation to Age, Growth, and Condition in Four Species of Fishes from Clay Lake, Ontario

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SCOTT, D. P. 1974. Mercury concentration of white muscle in relation to age, growth, and condition in four species of fishes from Clay Lake, Ontario. J. Fish. Res. Board Can. 31: 1723–1729.

Studies of large (about 125) samples of walleye (*Stizostedion vitreum*), northern pike (*Esox lucius*), white sucker (*Catostomus commersoni*), and lake whitefish (*Coregonus clupeaformis*) from each of four areas of Clay Lake, Ontario, a highly mercury-contaminated lake, have confirmed previous findings that the larger the fish, the greater the white muscle mercury concentration, within species, within populations. This relationship was statistically broken into separate age:concentration and growth:concentration partial regressions; older fish and faster growing fish were generally more contaminated, subject to the above species–area restrictions. However, the generally positive correlation between concentration and condition (as measured by relative heaviness of individuals within area–species samples) found in the previous study is here contradicted; relatively heavier fish tended to have lower Hg concentrations. Despite the relatively small size of the lake (about 11 × 2 km) analyses of multiple covariance indicated profound within-species differences between the samples from the four areas. However, there did not appear to be any obvious correlation between these differences and sediment mercury values.

The various statistical relationships do not appear to be simple, first-order regressions; rather, there appear to be significant interactions between age and growth, and age and condition, which tend to change the partial slopes with age. The four species were distinctly different in most of their relationships.

SCOTT, D. P. 1974. Mercury concentration of white muscle in relation to age, growth, and condition in four species of fishes from Clay Lake, Ontario. J. Fish. Res. Board Can. 31: 1723–1729.

Des études effectuées sur de grands échantillons (ca 125) de dorés (*Stizostedion vitreum*), grands brochets (*Esox lucius*), meuniers noirs (*Catostomus commersoni*) et grands corégones (*Coregonus clupeaformis*) provenant de chacune de quatre régions du lac Clay,

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Ontario, un lac fortement contaminé par le mercure, corroborent des constatations antérieures, à savoir que plus le poisson est grand, plus forte est la teneur en mercure du muscle blanc, au sein d'une même espèce et au sein d'une même population. Nous avons subdivisé par des méthodes statistiques cette relation en régressions partielles distinctes âge: concentration et croissance:concentration; les poissons âgés et les poissons à croissance rapide sont généralement plus contaminés, sous réserve des restrictions espèces–régions mentionnées plus haut. Cependant, la corrélation généralement positive entre la concentration et l'état d'embonpoint (mesuré en poids relatif des individus des échantillons intrarégionaux et intraspécifiques), obtenue lors d'une étude précédente, ne tient plus ici; les poissons relativement plus lourds ont tendance à avoir de plus faibles concentrations de Hg. Malgré l'étendue relativement petite du lac (environ 11 × 2 km), les analyses de covariance multiple indiquent de grandes différences intraspécifiques entre les échantillons provenant des quatre régions. Cependant, il ne semble pas y avoir de corrélation évidente entre ces différences et la teneur en mercure du sédiment.

Les diverses relations statistiques ne semblent pas être des régressions de premier ordre simples; il semble plutôt y avoir interactions significatives entre l'âge et la croissance, et entre l'âge et l'état d'embonpoint, interactions qui tendent à modifier les pentes partielles suivant l'âge. Les quatre espèces diffèrent nettement dans la plupart de leurs relations.

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In a previous paper (Scott and Armstrong 1972) a generally positive correlation between mercury concentration in white muscle and fish length was demonstrated in a number of species of freshwater fishes from Manitoba and northwestern Ontario. A more variable positive correlation was shown between mercury concentration and condition (fatness). Among the samples, those from Clay Lake, Ontario were by far the most heavily contaminated. However, of the four species examined from this lake, two showed nonsignificant relationships ($P < .9$) with either length or condition (walleye, *Stizostedion vitreum*, and lake whitefish, *Coregonus clupeaformis*). Northern pike (*Esox lucius*) showed a highly significant ($P > .99$) relationship between mercury concentration and length but not condition, whereas white sucker (*Catostomus commersoni*) had significant ($.9 \leq P < .99$) relationships between mercury concentration and both length and condition.

The sample sizes (20–24) of that study were rather small, and the range of lengths and condition factors quite restricted since these were essentially samples of commercial catches. To obtain some further insight into these contamination relationships, much larger, wider-ranged samples of these four species were obtained from Clay Lake in August, 1972, and the statistical relationships between mercury concentration and age (not previously considered), growth, condition, and area of Clay Lake were examined.

Materials and Methods

Standards gangs of gillnets were used to collect samples of walleye, pike, white suckers, and whitefish in the four areas of Clay Lake shown in Fig. 1 (modified from Armstrong and Hamilton 1973) in August, 1972. Fork lengths (L — centimeters), total weights (grams), and scale samples were taken, and specimens of white muscle preserved for mercury analyses. Considerable effort was expended to obtain the maximum possible size ranges among the samples:

Species	Scott and Armstrong (1972)		Present study	
	n	L range (*cm*)	*n*	L range (*cm*)
Walleye	24	33.0–50.0	508	15.3–71.1
Pike	25	36.0–62.0	497	21.2–80.3
White sucker	20	26.0–48.0	479	14.6–49.6
Whitefish	20	19.0–53.0	234	25.7–52.5

The paucity of whitefish in areas 1 and 3 (Fig. 1) appears to have been due to the approach of the spawning season as the main spawning grounds are in areas 2 and 4. The method of Armstrong and Uthe (1971) was used to determine mercury concentration; age of each fish was determined from the scales.

Statistical analysis of the data proceeded in several stages:

1. Where possible, within-sample, within-age regressions of mercury concentration on lengths and condition were fitted, as in the previous paper (Scott and Armstrong 1972). Analyses of multiple covariance showed immediately that between-age differences existed in several within-species, within-areas samples.
2. Because it appeared that, at least in some cases, there was a progression of slopes of the partial regressions with age, the following model was fitted, to take into account absolute exposure time (age), relative growth (length/E[length]), and relative condition (weight/E[weight]):

$$Y = \beta_0 + \beta_{11}X_1 + \beta_{12}X_2 + \beta_{12}X_1X_2 + \beta_{33}X_3 + \beta_{13}X_1X_3 \quad (1)$$

where $Y = \log_{10}$ (mercury concentration in ppm in white muscle);

X_1 = age in whole years (i.e. number of annuli on scales);

$X_2 = \log_{10}$ (length/E[length]) $= \log_{10}$ (relative growth) within samples;

$X_3 = \log_{10}$ (weight/E[weight]) $= \log_{10}$ (relative condition) within samples;

E(length) was determined from within-sample regressions of $\log_{10}$ (length) on age, and E(weight) from within-sample regressions of $\log_{10}$(weight) on age and $\log_{10}$(growth). These logarithmic transformations of length and weight into relative growth and relative condition, respectively, decorrelate the so-called independent variables and permit simple tests of significance of the least squares regression coefficients. There are still, however, some strong correlations between the simple variables and the interaction product terms; tests of significance were therefore carried out on the two variable pairs, growth and age–growth, and condition and age–condition, by the usual reduction-in-error-variance methods (Snedecor 1946).

For clarity, this may be shown as a factored version of the model:

$$Y = (\beta_0 + \beta_{11}X_1) + (\beta_{22} + \beta_{12}X_1)X_2 + (\beta_{33} + \beta_{13}X_1)X_3 \quad (2)$$

In this manner, the model reduces to a simple trivariate plane at any specific age, the functions within parentheses becoming scalars. For example, at age 0, $\beta_{11}X_1$, $\beta_{12}X_1$, and $\beta_{13}X_1$ all vanish, leaving the equation

$$Y = \beta_0 + \beta_{22}X_2 + \beta_{33}X_3 \quad (3)$$

The signs of β_{12} and β_{13} govern whether the partial slope of Y on X_2 and Y on X_3 flatten or steepen from their initial values as the fish grow older. Several other factoring combinations and sequential transformations are possible, and some of the more obvious were tested. However, none fitted as consistently well as this version.

3. Analyses of simple and multiple covariance (after Mood 1950) were used to determine whether there were within-species, between-areas differences in adjusted group means (ad $\overline{X}_j$) and partial regression coefficients ($\hat{b}_{jk.i}$) and whether weighted mean partial regression coefficients for each species differed from zero ($\hat{b}_{jki.w} = 0$), as in Scott and Armstrong (1972). Bartlett's (1937) test was used to indicate possible nonhomogeneity of variance within species, although results of this test must be viewed with suspicion. Standard methods (Dixon and Massey 1957) were used to eliminate outliers.

Results

The choice of model is difficult in investigations of this kind due to the many significant age–size–condition correlations which naturally exist. The

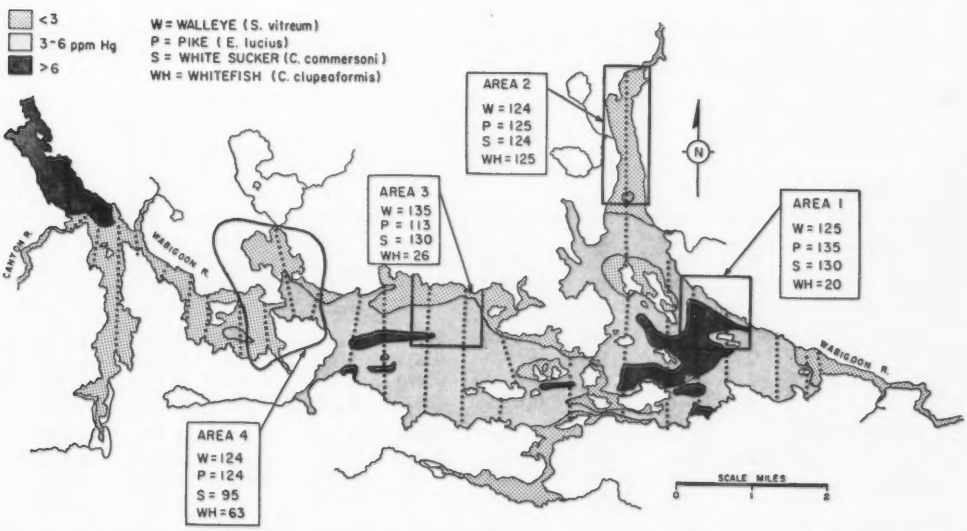

FIG. 1. Clay Lake, Ontario, indicating a) bottom sediment mercury content contours (after Armstrong and Hamilton 1973), and b) sample areas and numbers of samples of each species captured.

transformations used here are biologically logical in that 1) age is an estimate of chronological time of exposure of the fish to contamination, 2) relative growth, as defined, is a measure of physiological time of exposure or total metabolism, relative to other members of that area–species group, and 3) relative condition, as defined, is a measure of metabolic efficiency, i.e. the relationship between total food intake and requirements for maintenance and growth, again relative to other members of the group.

The results listed in Table 1–4 may be summarized as follows:

1. In every sample analyzed, there is an extremely significant ($P > .999$), positive relationship between age and mercury concentration in the white muscle. In addition, the regression coefficients are similar in value, the ratio between largest and smallest being less than 2.6–1 (Table 2).

From the results, chronological time is certainly effective in all samples; the longer the exposure the greater the mercury concentration, independent of other factors.

2. The compound relationship between growth ($\hat{b}_{22}$ and $\hat{b}_{12}$) and mercury concentration is highly variable both within and between species, although 12 of the 14 samples show at least high significance ($P > .99$). Three patterns emerge: in walleye, the linear part (i.e. $\hat{b}_{22}$, the value at age zero) is somewhat positive, and the combination becomes progressively more positive as age increases (i.e. $\hat{b}_{12}$ is positive). In pike, by contrast, the linear part is strongly positive but the combination becomes less so with age; even though within-sample values of $\hat{b}_{12}$ are not significant, the weighted mean value is so. In the white sucker, the linear part is generally negative or nonsignificant, but there is a very strong increase in slope with age. Whitefish from area 2 show the sucker pattern significantly, whereas those from area 4 are similar to pike although not statistically significant.

With growth, as measured within-samples, the complex relationship likewise indicates that physiological time of exposure is generally positively correlated with mercury concentration. Where the compound slope decreases with age, it appears to reach zero rather late, e.g. pike, area 1, approximately 16 yr ($2.2205 + (16 \times -0.1411) = -0.0371$). Since the oldest pike netted was 10 yr old, the positive relationship appears to hold for the majority of the populations. In suckers, although the slope is negative at early ages in three of four areas, this becomes positive very early and the general rule holds for the populations.

3. The compound relationship between condition ($\hat{b}_{33}$ and $\hat{b}_{13}$) and mercury concentration is also highly variable.

It is much more difficult to generalize about the complex relationship between condition, as defined within-samples, and mercury concentration.

TABLE 1. Within-sample decorrelation equations used to derive growth factor, $X_2 = \log_{10}(L/E(L))$ and condition factor, $X_3 = \log_{10}(W/E(W))$ where L = fork length (cm) and W = weight (g). Significance of all slope coefficients — $P > .999$.

			Regression of $\log_{10}$ (L) on age (X_1)			Regression of $\log_{10}$ (W) on age (X_1) and X_2			
Species	Area	N	$\hat{b}_0$	$\hat{b}_1$	$\hat{r}^2$	$\hat{b}_0$	$\hat{b}_1$	$\hat{b}_2$	$\hat{R}^2$
Walleye	1	103	1.403	0.0477	0.778	2.273	0.145	2.906	0.974
	2	76	1.456	0.0393	0.705	2.425	0.123	3.097	0.982
	3	128	1.455	0.0302	0.594	2.377	0.100	3.602	0.971
	4	119	1.565	0.0130	0.242	2.792	0.037	3.132	0.922
Pike	1	135	1.484	0.0576	0.711	2.278	0.177	3.166	0.973
	2	122	1.504	0.0503	0.631	2.371	0.148	2.824	0.974
	3	106	1.461	0.0546	0.690	2.240	0.166	2.901	0.987
	4	121	1.464	0.0673	0.632	2.247	0.199	2.969	0.984
White sucker	1	125	1.316	0.0689	0.644	2.072	0.218	2.978	0.972
	2	124	1.363	0.0537	0.446	2.241	0.167	2.954	0.964
	3	128	1.347	0.0484	0.555	2.232	0.145	2.855	0.961
	4	95	1.292	0.0653	0.638	2.034	0.203	2.986	0.988
Whitefish	2	122	1.385	0.0389	0.567	2.298	0.118	2.578	0.897
	4	50	1.423	0.0346	0.434	2.317	0.128	3.175	0.962

TABLE 2. Multiple regressions of mercury/age/growth/condition relations for four species of fish from four areas of Clay Lake, Ont. Regression coefficients as follows: $\hat{b}_0$ = intercept; $\hat{b}_{11}$ = partial coefficient for age; $\hat{b}_{22}$ = part. coef. for growth factor; $\hat{b}_{12}$ = part. coef. for interaction between age and growth; $\hat{b}_{33}$ = part. coef. for condition factor; $\hat{b}_{13}$ = part. coef. for interaction between age and condition. Significance by superscript: 0 is $P < 0.9$; 1 is $0.9 \leq P < 0.99$; 2 is $0.99 \leq P < 0.999$; 3 is $0.999 \leq P$. See text for explanation of growth and condition factors. ad $\overline{X}$'s are adjusted group means.

Species	Area	N	$\hat{b}_0$	$\hat{b}_{11}$	$\hat{b}_{22}$	$\hat{b}_{12}$	$\hat{b}_{33}$	$\hat{b}_{13}$	$\hat{R}^2$	s^2	ad $\overline{X}$ (log_{10})	ad $\overline{X}$ (ppm)
Walleye	1	103	0.3245	0.1259^3	$(0.2135^0$	$0.4573^3)^3$	$(0.5621^0$	$-0.2423^1)^1$	0.8776	.006690	0.8892	7.748
	2	76	0.4649	0.0716^3	$(1.8378^0$	$-0.3430^0)^0$	$(2.3644^0$	$-0.8840^1)^1$	0.4593	.018316	0.7861	6.110
	3	128	0.4764	0.0765^3	$(0.7046^1$	$0.1421^0)^3$	$(1.6742^2$	$-0.3333^2)^1$	0.7336	.009264	0.8197	6.602
	4	119	0.6374	0.0556^3	$(1.2851^1$	$0.0356^0)^3$	$(1.2187^0$	$-0.3418^1)^3$	0.6689	.005521	0.8870	7.708
	weighted mean	–	–	0.0805^3	0.7411^1	0.1683^1	1.0638^2	-0.3390^3	–	–	–	–
Pike	1	135	0.5338	0.0838^3	$(2.2205^3$	$-0.1411^0)^3$	$-(0.8831^1$	$-0.5676^3)^3$	0.6921	.012280	0.8051	6.384
	2	122	0.5000	0.0824^3	$(2.0302^3$	$-0.0723^0)^3$	$(-0.9246^0$	$-0.0305^0)^2$	0.5749	.017240	0.7671	5.849
	3	106	0.3776	0.0912^3	$(2.4640^3$	$-0.1524^0)^3$	$(-0.9856^0$	$0.0581^0)^1$	0.8003	.008276	0.6732	4.711
	4	121	0.4539	0.1154^3	$(1.1036^2$	$-0.0593^0)^3$	$(-0.7071^0$	$0.0784^0)^1$	0.7391	.010206	0.8258	6.723
	weighted mean	–	–	0.0929^3	1.8686^3	-0.1051^1	-0.0824^0	-0.1950^1	–	–	–	–
White sucker	1	125	–0.1635	0.1415^3	$(-0.1357^0$	$0.5122^2)^3$	$-(0.5724^0$	$-0.1389^0)^0$	0.7088	.008341	0.3962	2.490
	2	124	–0.0924	0.1033^3	$(0.3988^0$	$0.5403^1)^3$	$(-0.1881^0$	$0.0212^0)^0$	0.5121	.017819	0.3162	2.071
	3	128	–0.0941	0.0924^3	$(-1.7671^1$	$0.8331^3)^3$	$-(0.7485^0$	$-0.1558^0)^0$	0.5362	.009275	0.2709	1.866
	4	95	–0.0222	0.0880^3	$(-0.1216^0$	$0.4024^1)^3$	$(-0.1222^0$	$-0.1440^0)^0$	0.4144	.022448	0.3261	2.119
	weighted mean	–	–	0.1040^3	-0.2031^0	0.5220^3	0.4139^0	-0.1085^0	–	–	–	–
Whitefish	2	122	–0.3860	0.1083^3	$(-3.1656^1$	$0.9389^2)^2$	$(-2.0461^0$	$-0.4631^0)^0$	0.3086	.032910	0.1236	1.329
	4	50	–0.1529	0.1359^3	$(1.7146^0$	$-0.1782^0)^0$	$(4.5300^0$	$-0.7910^0)^0$	0.5170	.021552	0.4863	3.064
	weighted mean	–	–	0.1144^3	-2.3271^1	0.7345^1	2.2353^1	-0.4962^1	–	–	–	–

Individual regression coefficients are generally less significant than the growth coefficients, but a pattern does exist. Where a significant compound slope is shown (i.e. walleye and pike), the slope decreases generally with age or is already negative. For walleye, ages for zero slope for the four areas are 2.3, 2.7, 5.0, and 3.6, the relationship being negative at all older ages. For pike, the relationship is less consistent; area 1 pike have zero slope at 1.5, area 2 pike have a negative slope throughout life, and areas 3 and 4 populations are reversed in having negative slopes which gradually increase (i.e. becoming more positive). The annual increments for these last two are, however, not statistically significant, and pike samples generally can be said to demonstrate negative slopes of mercury concentration on condition. The remaining groups are quite erratic. It should be noted that 11 of 14 values of $\hat{b}_{13}$ are negative, as are all four values of weighted means. It seems likely that the slope of mercury concentration on condition does diminish with age. Generally, then, relatively heavier fish tend to have lower Hg concentrations and this tendency becomes more pronounced with age.

4. Analyses of multiple covariance, Table 3–4, show there to be extremely significant differences in within-species, between-areas values of most regression coefficients, adjusted group means, and residual variances ($P > .999$ in 22 of 28 tests) and some significance in all but one of the remainder. Despite the evidence in Table 2 that the differences are numerically small, large sample size and range permit these small differences to be judged real. The only obvious pattern of differences between samples from different areas appears in the adjusted group mean mercury concentrations (i.e. average within-species, mean age, mean growth, mean condition Hg concentration) where, for all species, values for areas 1 and 4 are higher than those for areas 2 and 3. From Fig. 1, higher values in area 1 seem consistent with bottom sediment concentrations but this is not so in area 4.

5. Tests of the significance of weighted mean regression coefficients generally back up the results from the individual tests that age and growth (both parts) strongly influence white muscle mercury concentration, but the evidence for condition effects is much less strong.

TABLE 3. Analyses of multiple covariance and tests for homogeneity of residual variance for within-species, between-areas comparisons of multiple regressions of mercury/age/growth/condition relations from Clay Lake, Ont. Variables and probability levels as in Table 2.

	"F" for equality of $\hat{b}$'s						"χ^2/df" for homoscedasticity		"F" for equality of ad $\bar{X}$'s	
Species	df	$\hat{b}_{11}$'s	$\hat{b}_{22}$'s	$\hat{b}_{12}$'s	$\hat{b}_{33}$'s	$\hat{b}_{13}$'s	df	s^2's	df	ad $\bar{X}$'s
Walleye	3;402	279.2^3	14.21^3	49.24^3	14.51^3	16.75^3	3	11.36^3	3;417	7.773^3
Pike	3;460	87.59^3	66.13^3	4.952^2	70.98^3	106.7^3	3	5.373^3	3;475	34.88^3
White sucker	3;448	112.8^3	39.28^3	28.50^3	4.132^2	2.674^1	3	12.61^3	3;463	18.18^3
Whitefish	1;160	11.30^3	40.09^3	46.65^3	5.354^1	2.757^1	1	2.648^0	1;165	133.1^3

TABLE 4. Analyses of covariance for species-weighted mean partial regression coefficient equality with zero. Species, variables, and probability levels as in Table 3.

	"F" for equality of weighted mean $\hat{b}$'s and zero					
Species	df	$\hat{b}_{11}$	$\hat{b}_{12}$	$\hat{b}_{12}$	$\hat{b}_{33}$	$\hat{b}_{13}$
Walleye	1;402	124.1^3	6.467^1	6.240^1	10.16^2	20.36^3
Pike	1;460	468.1^3	70.72^3	3.019^1	0.0650^0	4.457^1
White sucker	1;448	232.3^3	0.2908^0	28.07^3	0.3556^0	0.4836^0
Whitefish	1:160	50.11^3	2.820^1	5.940^1	2.713^1	3.064^1

Discussion

The present results are consistent with the general pattern found by Scott and Armstrong (1972) in that mercury contamination of fish increases with size; older, larger fish have greater mercury concentration in white muscle. The previous general finding that relative condition and mercury concentration were positively correlated is not supported here; it is tempting to speculate that the high mercury values in Clay Lake may be the cause and condition the effect such that high mercury depresses condition. The relationship is strongest in the two piscivores and, since methyl mercury (the form in which most of the mercury in fish is found) is known to be a neurotoxin, (Diamond and Sleight 1972) these levels may be high enough to inhibit their hunting ability significantly. The facts that the relationship of concentration with growth and condition are not constant with age but seem to be both species- and area-dependent indicate the need for detailed physiological experiments on mercury uptake and loss under various environmental conditions.

Finally, the effects of sample size and range are clearly visible when compared with the results shown for these species in Clay Lake in Scott and Armstrong (1972). The objective stated in the earlier work of finding out whether satisfactory predictive relationships could be developed was clearly not satisfied in the small walleye and whitefish samples. In the present study, the fact that all 14 groups show high age and growth correlations demonstrate that such predictions are possible, given adequate sample numbers and ranges from which to develop the predictive equations.

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Swimming Speeds of Yellow Perch (*Perca flavescens*) Following an Abrupt Change in Environmental Temperature

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OTTO, R. G., AND J. O'HARA RICE. 1974. Swimming speeds of yellow perch (*Perca flavescens*) following an abrupt change in environmental temperature. J. Fish. Res. Board Can. 31: 1731–1734.

Groups of yellow perch (*Perca flavescens*) were acclimated to constant temperatures of 10 and 20 C. Acclimation temperatures were then abruptly reversed. Critical swimming speeds were determined prior to and following the temperature change at 24-h intervals. Adjustment (acclimation) of fish subjected to an increase in temperature was immediate. Fish subjected to a decrease in temperature required less than 24 h to regain maximum swimming capability.

OTTO, R. G., AND J. O'HARA RICE. 1974. Swimming speeds of yellow perch (*Perca flavescens*) following an abrupt change in environmental temperature. J. Fish. Res. Board Can. 31: 1731–1734.

Des groupes de perchaudes (*Perca flavescens*) ont été acclimatés à des températures constantes de 10 et 20 C. Les températures d'acclimatation ont été ensuite brusquement interverties. Les vitesses maximales de nage soutenue ont été déterminées avant et après les changements de température à intervalles de 24 h. L'ajustement (acclimatation) des poissons soumis à une augmentation de température a été immédiate. Les poissons soumis à un abaissement de température prirent moins de 24 h à regagner leur aptitude à une vitesse de nage maximale.

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ADAPTATION to changing temperatures can be subdivided temporally into several aspects. Most rapid is that process termed accommodation, occurring in seconds or minutes as the threshold of an excitable tissue rises in conformance with an increased level of stimulus (Fisher 1958). Shifts in whole animal response to environmental temperature changes (acclimation) are somewhat slower, typically requiring days or weeks (Roberts 1967). Slowest of all are gradual changes in population or species characteristics in response to long-term shifts in environmental temperatures (Somero et al. 1968). Such changes comprise the evolutionary process and are frequently measured in geologic time units.

Previous investigators have considered the effects of changing temperatures on a wide variety of criteria (Brett 1971; Fry 1971; Hochachka and Somero 1971). The majority of such studies have been concerned with steady state or homoiostatic conditions existing after the time course of individual adaptation was completed. Consideration of transient changes in whole animal response occurring during the time interval separating steady state conditions has been restricted primarily to the determination of tolerance limits (Brett 1944; Allen and Strawn 1971) and various measures of metabolic rate or oxygen consumption (Auerbach 1957; Klicka 1965). The nature of such transient changes in response is of particular interest for fishes occupying habitats in which rapid, short-term temperature fluctuations occur routinely. This report describes the time course of individual adaptation to a rapid change in temperature for a eurythermal fish in terms of its ability to swim or perform work.

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Materials and Methods

APPARATUS

Performance capabilities were determined using a stamina tunnel similar in design to that employed by Griffiths and Alderdice (1973). The apparatus was an open loop of some 1110-liter capacity. Water was pumped into a fiberglass pipe from a stainless steel header box by a propeller hooked to a 3/4 hp variable speed DC motor. The water was forced through screen into a 12.7 cm diameter clear plexiglass swimming

chamber approximately 50 cm in length. At the rear of the chamber, three graphite rings were connected to a low voltage AC source to provide a shock stimulus to the fish.

Behind the rings, where the tunnel emptied into the header box, either a screen or chute could be fitted over the end of the tunnel. The header box contained a chilling coil and three 4000-W heaters. When operated in unison, these heaters were capable of raising the water temperature 1 C each 2 1/2 min. Rapid chilling could be achieved by adding ice to the header box. One of the heaters could be operated independently with a thermostat to provide precise temperature control. Speeds up to 100 cm/s could be achieved. Water velocities in cm/s were read directly from an electronic tachometer attached to the propeller drive. Calibration was performed with a pitot-tube manometer.

Procedure

Groups of 10 yellow perch ranging in standard length from 9 to 10 cm were maintained in 150-liter fiberglass tanks at 10 and 20 C for 30 days. Test groups of five fish were then randomly selected from an acclimation category, placed in the tunnel at the acclimation temperature, and maintained for 30 min at a water velocity of 5 cm/s. Water velocity was then increased in increments of 5 cm/s at 15-min intervals. Individual fish were removed from the tunnel as they fatigued.

This test sequence was continued for both acclimation groups at 24-h intervals for 4 days to allow the fish to adjust to the procedure. On the fifth day, acclimation temperatures were reversed, 10 C fish being moved to 20 C and vice versa. The temperature change was imposed while the fish were in the stamina tunnel during the 30-min pre test period. Fish were tested immediately following the reversal of acclimation temperatures and at 24-h intervals thereafter for 6 days.

Critical swimming speed for each fish was calculated according to the formula:

$$\text{Critical Speed} = \text{Last Recorded Speed} + \text{Time at New Speed Increment} \times \text{Increment of Speed}$$

as described by Brett (1967). Median values for each test group were estimated graphically by plotting the logarithms of individual critical speeds against the cumulative percentage of fish fatigued in probit units. The systematic small sample error inherent in this procedure was corrected as described by Bliss (1937).

Results and Discussion

Perch acclimated to 10 and 20 C had median critical swimming speeds of 15.5 cm/s and 25.2 cm/s, respectively, on the first day of testing (Fig. 1). These speeds increased to 21.0 cm/s for 10 C-acclimated fish and 33.0 cm/s for 20 C fish on the second day. Performance levels for both acclimation groups then remained stable for the remainder of the 4-day training period. The improvement in swimming performance resulting from experience for 20 C fish was approximately twice that for 10 C fish.

Since a random process was employed to select the five test fish from the 10-fish acclimation group, test groups after day one might have contained both naive and experienced fish. However, variability of response as indicated by slopes of the probit distributions remained approximately the same throughout the 10-day test period, suggesting a group effect wherein the presence of experienced individuals improved performance of naive fish (Fig. 2).

An immediate increase in performance was observed following an increase in water temperature

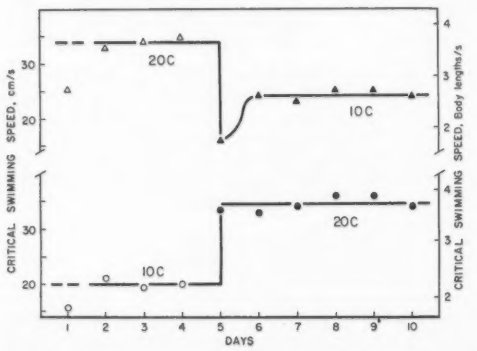

FIG. 1. The effects of an abrupt change in temperature on the maximum sustained swimming speed of the yellow perch (*Perca flavescens*). The time of temperature change is indicated by arrows.

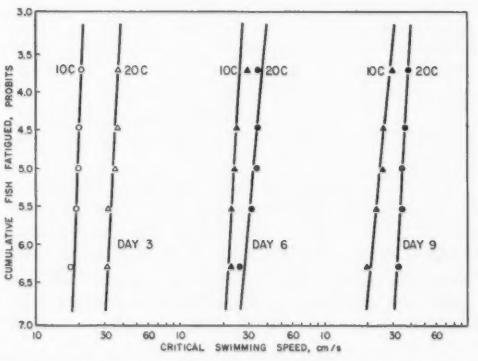

FIG. 2. Sample probit plot showing results for individual tests. Symbols are as in Fig. 1.

from 10 to 20 C. Mean critical swimming speed was raised to 33.5 cm/s, a gain of 12.5 cm/s. This value is comparable to the swimming speed of fish acclimated to 20 C for 30 days. Performance levels for this group remained stable for the remainder of the 10-day period. Upper and lower 95% confidence limits for the stabilized mean critical swimming speed were 36.9 cm/s and 32.0 cm/s, respectively.

Fish transferred directly from 20 to 10 C exhibited a loss of performance capability. Mean swimming speed decreased to 15.5 cm/s, a loss of 17.5 cm/s. Swimming speed for this group then increased to 24.5 cm/s at the end of 24 h, remaining at this level for the remainder of the test period. Upper and lower 95% confidence limits for the stabilized mean critical swimming speed were 26.3 cm/s and 22.9 cm/s, respectively.

The stable performance level for fish transferred from 20 to 10 C (24.5 cm/s) was significantly higher than anticipated from swimming speeds observed for fish acclimated to 10 C only (20.0 cm/s). The difference corresponds with the greater increase in performance associated with experience in the tunnel for 20 C-acclimated fish when compared with 10 C-acclimated fish. However, the physiological (or behavioral) basis for this difference in response remains obscure.

In evaluating our results, it is important to recognize the complexity of temperature adaptation where different rates of application and levels of temperature may be involved in conjunction with seasonal, size, age, and species differences. In general, our results contrast with studies on rates of change in tolerance following abrupt changes in temperature. For example, the opaleye (*Girella nigricans*) required 1 day to complete 90% acclimation of heat resistance following a temperature increase from 14 to 26 C (Doudoroff 1942). Fifteen days were required for a 90% loss of cold resistance. When the temperature change was reversed, more than 25 days were required for a 90% loss of heat resistance with a similar time needed for 90% gain in cold resistance. Similar examples are given by Brett (1971) for a number of species.

Our results are in somewhat better agreement with observations on rates of change in metabolic rate (oxygen consumption) following temperature changes. For example, Klicka (1965) found that for the goldfish (*Carassius auratus*) stable rates of oxygen consumption were regained in 2–5 days following an increase or decrease in temperature of 15 C.

Ecological implications of our results are apparent. Yellow perch are commonly found in shallow lake waters where rapid temperature fluctuations occur, particularly during late spring and summer months. For example, in Lake Michigan (the source of fish for this study), stratification occurs during spring and summer and temperatures as high as 21–22 C are typical in inshore waters. Upwelling conditions associated with offshore winds frequently result in temperature fluctuations of the order employed in this study occurring over short (<6 h) periods of time.

The abilities of fishes resident in a thermally unstable environment of this nature to avoid predation, capture prey, and maintain "normal" activities are obviously dependent on the time course of adaptation. In the case of the yellow perch, at least, it appears that compensation for any adverse reduction in performance capabilities resulting from a sudden fluctuation in temperature is completed in an appropriately short time period.

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Biology of *Eubothrium salvelini* (Cestoda: Pseudophyllidea), a Parasite of Juvenile Sockeye Salmon (*Oncorhynchus nerka*) of Babine Lake, British Columbia

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BOYCE, N. P. J. 1974. Biology of *Eubothrium salvelini* (Cestoda: Pseudophyllidea), a parasite of juvenile sockeye salmon (*Oncorhynchus nerka*) of Babine Lake, British Columbia. J. Fish. Res. Board Can. 31: 1735–1742.

Adult worms recovered from naturally infected sockeye salmon (*Oncorhynchus nerka*) smolts provided the eggs for regeneration of *Eubothrium salvelini* in the laboratory. Of several copepods tested three species of *Cyclops* proved to be successful intermediate hosts whereby infections were ultimately transmitted to sockeye fry. The parasite's life cycle is described. The rate of development within the intermediate host is shown to be related directly to temperature. It is also demonstrated that susceptibility of fry to infection diminishes as they exceed a certain size.

BOYCE, N. P. J. 1974. Biology of *Eubothrium salvelini* (Cestoda: Pseudophyllidea), a parasite of juvenile sockeye salmon (*Oncorhynchus nerka*) of Babine Lake, British Columbia. J. Fish. Res. Board Can. 31: 1735–1742.

Des vers adultes prélevés sur des smolts de saumon nerka (*Oncorhynchus nerka*) infestés naturellement nous ont fourni des oeufs pour l'élevage d'*Eubothrium salvelini* au laboratoire. Parmi plusieurs copépodes étudiés, trois espèces de *Cyclops* se sont avérés des hôtes intermédiaires efficaces, grâce auxquels l'infestation a été finalement transmise à des alevins de saumon nerka. Nous décrivons le cycle biologique du parasite. Nous démontrons que le taux de développement dans l'hôte intermédiaire est directement relié à la température. Nous démontrons de plus que la susceptibilité des alevins à l'infestation diminue lorsqu'ils dépassent une certaine taille.

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BABINE Lake, 155 km long and 490 km^2 in surface area, is one of the largest lakes in British Columbia and a principal nursery area for British Columbia sockeye salmon (Larkin and McDonald 1968). Sockeye spawn in contributory streams and the outlet river between August and November; the fry enter the lake in the spring (May–June) and seaward migration of smolts occurs in May–June of the following year.

Dombroski (1955) reported approximately 30% of the 1952 and 1953 Babine smolt population to be infected with the intestinal cestode *Eubothrium salvelini* (Schrank 1790) and noted that the infected were smaller than uninfected smolts. Other investigators have found similar infection levels in subsequent years. More recently, Smith and Margolis (1970) and Smith (1973) have suggested that infected Babine sockeye smolts are of impaired vitality and may have a reduced chance of surviving to adulthood.

Concern for the effects of parasitization on Babine sockeye stocks, especially with the prospects of increased fry production in this system through the Babine Lake Salmon Development Program (Anon. 1968), has prompted investigation of *E. salvelini* and its effects on sockeye salmon production. Presented in this paper are results of studies on the life cycle of the parasite.

There has been considerable diversity in the usage of terms for describing various phases and stages of cestode development. I propose to use in this paper the terminology adopted in the review by Freeman (1973).

Materials and Methods

EGGS

Worms with gravid proglottids were obtained from sockeye salmon smolts trapped near the outlet of the

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lake and maintained for varying periods in either physiological saline or modified medium 199 (buffered TC med. 199, plus 10% foetal calf serum, 2% penicillin–streptomycin mixture) during which time numerous eggs containing oncospheres were released spontaneously. These eggs, transferred to fresh water and accumulated over a number of days, were used to infect intermediate hosts. For investigating the hatching process a trypsin–versene digest was tested for its effect on the embryophore. The digest consisted of trypsin, 0.5 g; versene (EDTA), 0.2 g; $NaHCO_3$, 1.1 g; phenol red (0.5 g %), 1 ml; $NaH_2PO_4 \cdot H_2O$, 0.07 g; KCl, 0.2 g; NaCl, 4.0 g; distilled water, 500 ml; pH adjusted to 7.2–7.4.

Intermediate Host

Attempts were made to infect the following copepods:

	Source
Cyclops scutifer Sars	Babine Lake
Cyclops bicuspidatus thomasi Forbes	Great Central Lake
Cyclops vernalis Fischer	Great Central Lake; Temporary ponds, Nanaimo
Macrocyclops fuscus Jurine	Temporary ponds, Nanaimo
Diaptomus novemdecimus M. S. Wilson	" " "
Diaptomus oregonensis Lilljeborg	Great Central Lake; Long Lake
Diaptomus sp.	Babine Lake
Epischura nevadensis Lilljeborg	Great Central Lake

All the above locations, except Babine Lake, are on central Vancouver Island, B.C. Samples from each collection were examined to establish absence of natural infection.

Lake copepods were exposed to high concentrations of *Eubothrium* eggs in 500-ml round-bottomed flasks, with frequent shaking to keep the eggs in suspension. Pond specimens that favored the bottom were placed with eggs in 200-ml cystallizing dishes with no shaking. Relative numbers of copepods and eggs varied and counts were not made routinely; on one occasion approximately 50,000 eggs and 1500 *C. scutifer* in 450 ml fresh water proved an effective exposure of copepods to infection. During exposure subsamples of 5–10 copepods were extracted periodically and examined at 540 ×. Exposure was terminated when at least 75% incidence of infection had been achieved.

The above procedures for maintenance of gravid worms and eggs and exposure of copepods to infection were performed at 5–6 C; thereafter, batches of infected copepods were usually maintained at 10 C (less frequently @ 5 + 15 C) in cylindrical battery jars and fed a mixed culture containing *Nannochloris* sp., *Asterionella* sp., and *Paramecium* sp. The development of worms to the infective metacestode stage was monitored through periodic sampling of these copepods. An inverted compound microscope was used for examinations at magnifications of 150 × –400 × with minimum injury to the copepods. Eggs and worms at various stages of development were isolated in saline or medium 199 and studied as wet mounts under a conventional compound microscope.

Final Host

The experimental final hosts were largely sockeye salmon fry reared from Babine Lake eggs at an experimental facility supplied with groundwater at temperatures of 5.7–8.0 C. In some cases the incubation of eggs and subsequent culturing of fry were conducted at lowered temperatures (3–4 C) to produce fry that were relatively late in hatching and small at the time of attempted infection.

For 3 days prior to exposure to infection the fry were isolated in small containers and conditioned to feed on copepods introduced from a Pasteur pipette. This procedure eliminated the need for force-feeding and facilitated the use of smaller fish as experimental hosts. In later experiments the infected copepods were placed within the carapace valves of live *Daphnia pulex* which were in turn fed to the fish as described above. This larger target elicited a prompter feeding response and made it easier to see when ingestion had occurred. Approximately 10 metacestodes were presented to each fish.

Following ingestion of infected copepods the fry were isolated in compartmented aquaria and fed frozen marine plankton, frozen brine shrimp, and live *Daphnia*. The *Daphnia* were determined free of natural cestode infection. Establishment of infection in fry was determined by later examination of the excised stomach, esophagus, pyloric caeca, and intestine at magnification 75 ×, with each organ pressed between a pair of 5-cm × 8-cm microscope slides.

Development of *E. salvelini* in fish at 10 C was observed in experimental fry for 43 days after infection. Supporting information and limited data on development past the 43rd day were obtained through observation of natural infections, i.e. fry captured after approximately 2 mo residence in Babine Lake were transferred to rearing facilities at Nanaimo and sampled periodically over the subsequent 12 mo.

Fixation, Staining Procedure

Eggs and worms in all stages of development were fixed in hot A.F.A. (aceto-formal-alcohol) and stained in Harris' haematoxylin, basic fuchsin, or eosin. Infected copepods were usually fixed, stained, and cleared intact, then dissected and the worms removed for mounting on slides. Eggs were deposited on slides that

were then coated with celloidin and taken through the staining and clearing process.

Results

The Egg

Mature worms were maintained for up to 30 days in physiological saline and more than 150 days in modified medium 199 at 5–6 C. Gravid segments appeared yellowish in contrast to the white of other segments. Eggs were discharged spontaneously through medially located uterine pores. Segments that were in the process of egg-shedding appeared swollen and expulsion of eggs was often accompanied by considerable body contortion in the region of the shedding segment. Spent segments survived in vitro several weeks after completion of egg-shedding, and remained attached to the rest of the worm for some time after death.

Eggs containing oncospheres (Fig. 1), spontaneously released, measured 88.75–102.51 × 48.96–71.91 μ, with shell thickness ca. 1.4 μ (n=15). Much smaller eggs (Fig. 2), with oncospheres not formed, 55.1–66.2 × 35.2–39.8 μ (n=15), were often discharged at the same time. Eggs containing oncospheres were found in the expelled feces of captive fish, suggesting this to be the normal means of passage of eggs into the water. Terminal segments were occasionally seen trailing from the anus and later dropping off, but as will be explained later, this phenomenon should not be mistaken for apolysis.

The egg is nonoperculate and its surface is smooth. The egg shell encloses the coracidium, which consists of the larva or oncosphere with its surrounding embryophore. The oncosphere (Fig. 1 and 3) is roughly pear-shaped, slightly motile, and equipped at one end with three pairs of hooks, each ca. 17.0 μ in length. No flame cells or penetration glands were seen in the oncosphere. The embryophore is wrinkled and nonciliated. The egg tends to sink in fresh water but can be kept in suspension with gentle agitation. Survival up to 30 days in fresh water at 5 C has been observed, with no evidence of spontaneous hatching.

"Hatching" induced with coverslip pressure was observed at 240 ×. It consisted of rupture of the outer shell immediately followed by release of the coracidium. In fresh water death followed rapidly; in physiological saline survival up to 2 h at ca. 8 C was observed. During this time the oncosphere was quite active but remained within the embryophore. When the liberated coracidium was placed in trypsin–versene digest, breakdown of the embryophore ensued shortly and the oncosphere was completely free in 35 min. The digest had no apparent effect on the intact egg.

Infection of the Intermediate Host

Of the crustaceans exposed to infection in the laboratory, only *Cyclops scutifer*, *C. bicuspidatus* and *C. vernalis* became readily infected. The susceptibility of *C. scutifer* and *C. bicuspidatus* to infection is size dependent. The data (Fig. 6) suggest that infection rates in *Cyclops* of prosome length of 500 μ or less may be expected to be low. Independent measurements of samples of *C. bicuspidatus* of Great Central Lake show this critical prosome length to correspond with a body length of approximately 870 μ, so that infection in this species is limited roughly to the stages copepodite IV to adult, according to the size–development relationship described by LeBrasseur and Kennedy (1972).

Cyclops scutifer and *C. bicuspidatus* constituted the bulk of copepods used as experimental intermediate hosts, *C. vernalis* being available only in small numbers. Two out of 2000 *C. scutifer* sampled May to July from Babine Lake were positive for naturally acquired *E. salvelini* infection; no such infections were found in 3000 *C. bicuspidatus* and 1200 *C. vernalis* sampled May–July from Great Central Lake where *E. salvelini* also occurs in juvenile sockeye salmon.

In the laboratory a 75% incidence of infection was usually achieved after 8–24 h exposure of adult and near-adult copepods to eggs at the concentrations described earlier. As the ratio of eggs to copepods diminished, longer periods of exposure were required. The intensity was usually one or two, less frequently three or four worms per copepod. Intensities in excess of four were sometimes encountered in the first 2 wk following exposure to infection, but were seldom observed in more advanced stages of infection.

The initial stages of infection in copepods were not observed directly. However, the common occurrence of ruptured egg capsules in the gut and free oncospheres in the gut and haemocoel of copepods directly after brief exposure to eggs suggests that hatching occurs during, or shortly after ingestion of the egg, and that oncospheres pass from the gut to the haemocoel not long after hatching.

There was no evidence of the embryophore in any of the freshly infected copepods. This covering, observed to remain intact in physiological saline, is presumably rapidly digested in the gut of the copepod. The effectiveness of the trypsin–versene digest on the coracidium suggests that digestive enzymes of the copepod may play a necessary part in the breakdown of the embryophore.

The newly hatched oncosphere observed in the gut of the copepod is very active and the hooks execute clawing movements. The actual passage from the gut to the haemocoel was not seen but the hooks appear quite capable of penetrating the gut wall, enabling entry of the worm directly into the haemocoel, provided the disposition of the worm in the gut permits the necessary contact. Entry is not always achieved and oncospheres that fail to reach the haemocoel have been seen to pass down the lumen of the gut to be ultimately voided. The frequent appearance of disembodied hooks in the gut indicates that the oncospheres are often digested.

Development in the Intermediate Host

The following account is based on observation of experimental infections in copepods cultured at 10 C.

Once established in the haemocoel of the copepod the oncosphere assumes the identity of a metacestode and development to the stage of caudate bothrio-plerocercoid progresses. Detached hooks are found occasionally in the haemocoel, indicating the demise and partial absorption of some metacestodes at various stages of development. Since organogenesis is not easily recognized during this stage of the life cycle, the main criteria of development are changes in shape, size (mainly length), cercomer and scolex formation. During its life in the copepod the larva grows from ca. 30–40 μ to 300–400 μ in length. The worm, at first round or pear-shaped upon hatching (Fig. 3), soon elongates to become cigar-shaped. After 15–20 days, the initial phase of cercomer formation, the attenuation of the posterior extremity of the worm up to a level slightly anterior to the hooks, is seen (Fig. 4). The attenuated part then becomes progressively pinched off from the rest of the body by a constriction that at first disappears and reappears with normal movements and during contraction and extension of the worm. This constriction becomes permanent and better defined with further development.

Infective Metacestode Stage

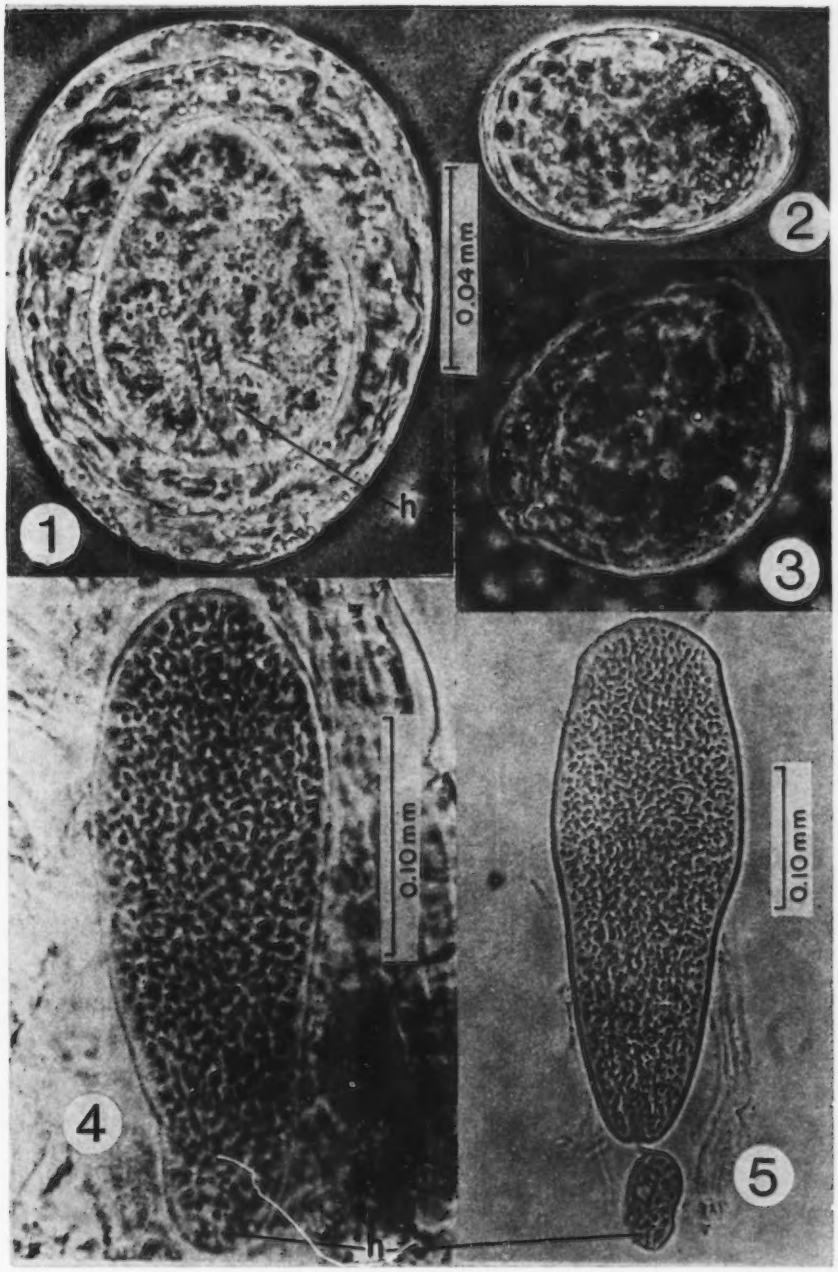
The peak of metacestode development in the copepod is apparently reached by the 25th–30th day (Fig. 5, 7). The worm, now quite active, is aligned either backwards or forwards with the gut of the copepod, and occupies most of the length of the haemocoel in the region of the cephalothorax, rarely protruding into the abdominal segments. The cercomer is attached to the main trunk of the worm by a very fragile stalk that is easily severed. The full complement of hooks remains on the cercomer, but in some cases the hooks have lost their terminal position and symmetrical arrangement. Independent activity of the hooks has apparently ceased, but the cercomer itself is capable of contractile movements. The cuticle of the main trunk of the worm is invaginated at the location of its attachment to the stalk of the cercomer, and the stalk is often fully withdrawn into this invagination, bringing cercomer and trunk together. At the anterior end of the body some development toward the characteristic bothriate holdfast is evident. As with the developing cercomer, the bothria at first appear and disappear with contraction and extension of the body (Fig. 10, 11, 12), later becoming permanent and well-defined.

The osmoregulatory system (Fig. 8), seen only in live specimens, appears to consist of four main vessels located dorsally and ventrally along each side of the main body of the worm. From these arise a complex array of delicate tubules that I found for the most part not traceable. Near the anterior end of the worm the main vessels join to form a ring from which more fine tubules continue anteriorly. Near the posterior end of the worm the vessels appear to come together presumably to join the excretory vesicle. The excretory vesicle was not discernible in my specimens, nor was the precise course of the terminal vessels and the flame cell pattern determined. However, up to 30 flame cells, including four in the cercomer, were seen in an individual worm. Calcareous corpuscles are in evidence throughout the worm including cercomer, with the exception of a clear area in the region of the scolex.

The advanced metacestode stage described above, and characterized superficially by the tenuous attachment of the cercomer, was for the following reasons presumed to be the stage infective to fish:

1. No further development in distinguishable characters was seen in worms that survived in copepods past this stage — up to 43 days at 10 C.
2. No cercomers were found in this study, or seen reported elsewhere, in even the youngest worms to appear in fish.
3. The appearance of this stage of metacestode was soon followed by a marked decline in abundance of infected copepods, suggesting the end of the copepod–metacestode association.
4. The main body of these worms, i.e. excluding the cercomer, essentially matched in appearance and size early stages of infection found in fish.

In order to examine the influence of temperature on development, comparisons were made among single-worm infections in *C. scutifer* maintained at constant temperatures 5, 10, and 15 C. Some obvious and consistent differences were observed



FIGURES 1–5.

FIG. 1. *Eubothrium salvelini* egg containing oncosphere (live); FIG. 2. Undeveloped egg (live); FIG. 3. Oncosphere: shortly after hatching (live); FIG. 4. 17-day metacestode in *C. scutifer* (permanent mount); FIG. 5. Infective metacestode excised from copepod (permanent mount). h = hooks.

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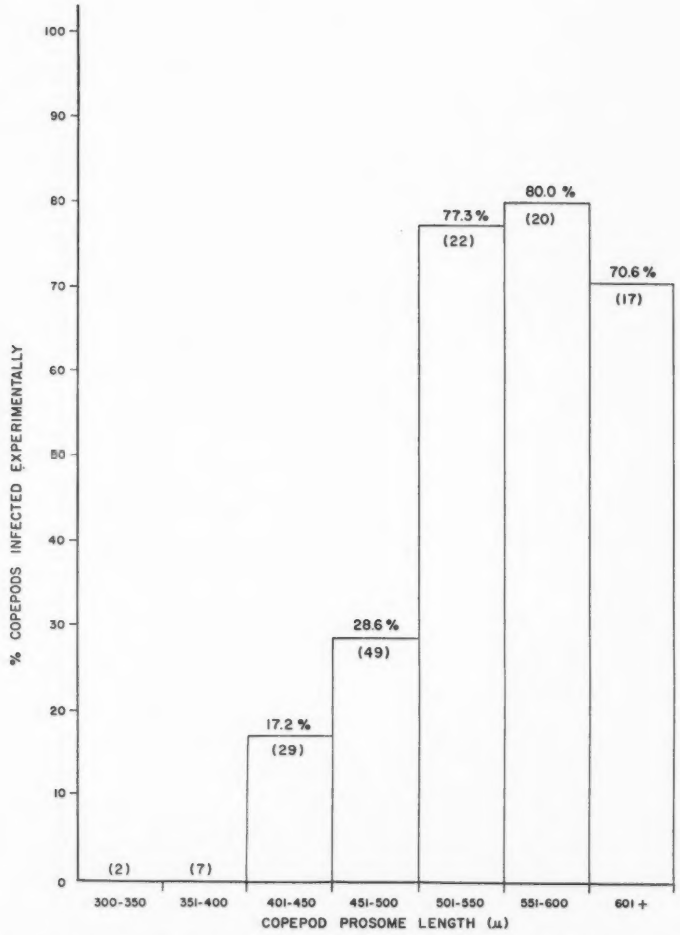
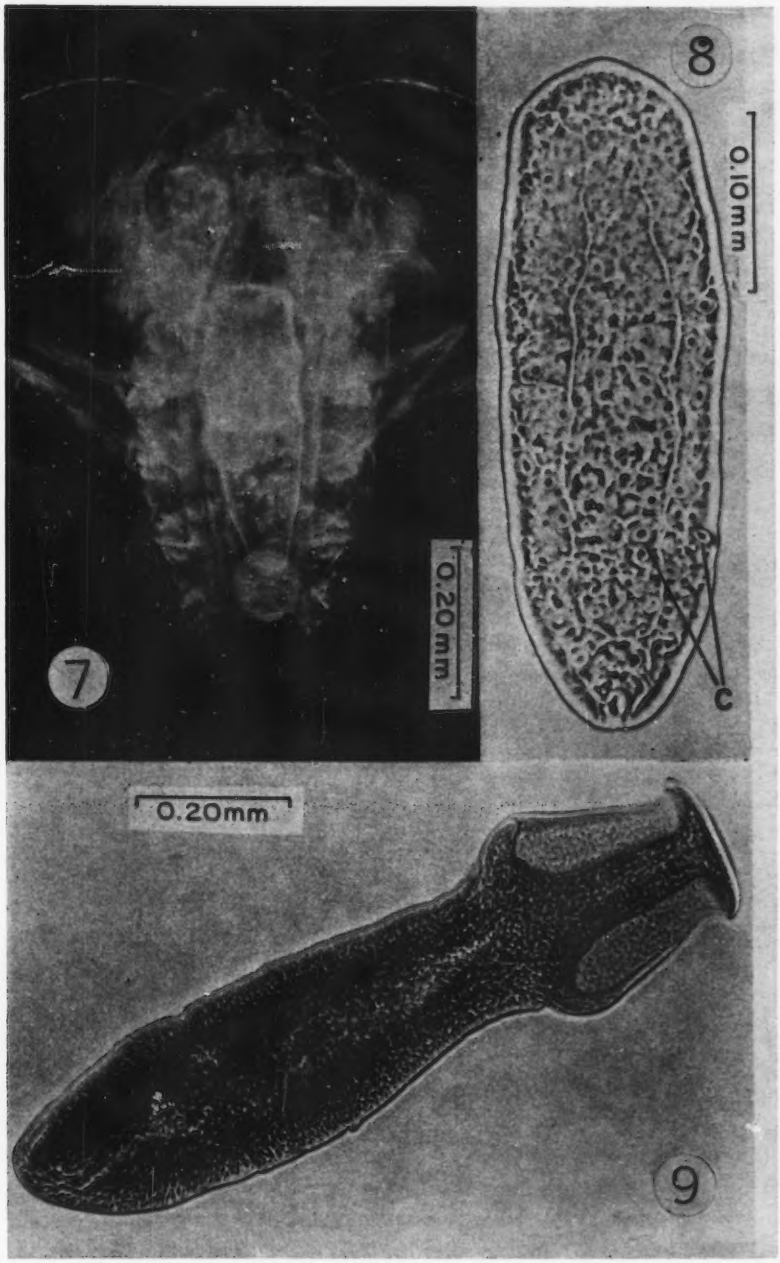


FIG. 6. *Cyclops bicuspidatus:* susceptibility to experimental infection vs. size. (Numbers in parentheses indicate sample size.)



FIGURES 7–9.

FIG. 7. Infective metacestode in *C. scutifer* (live); FIG. 8. Infective metacestode showing osmoregulatory system, calcareous corpuscles (live, cercomer detached); FIG. 9. Developing adult 30 days after introduction to fish (permanent mount). c = calcareous corpuscles.

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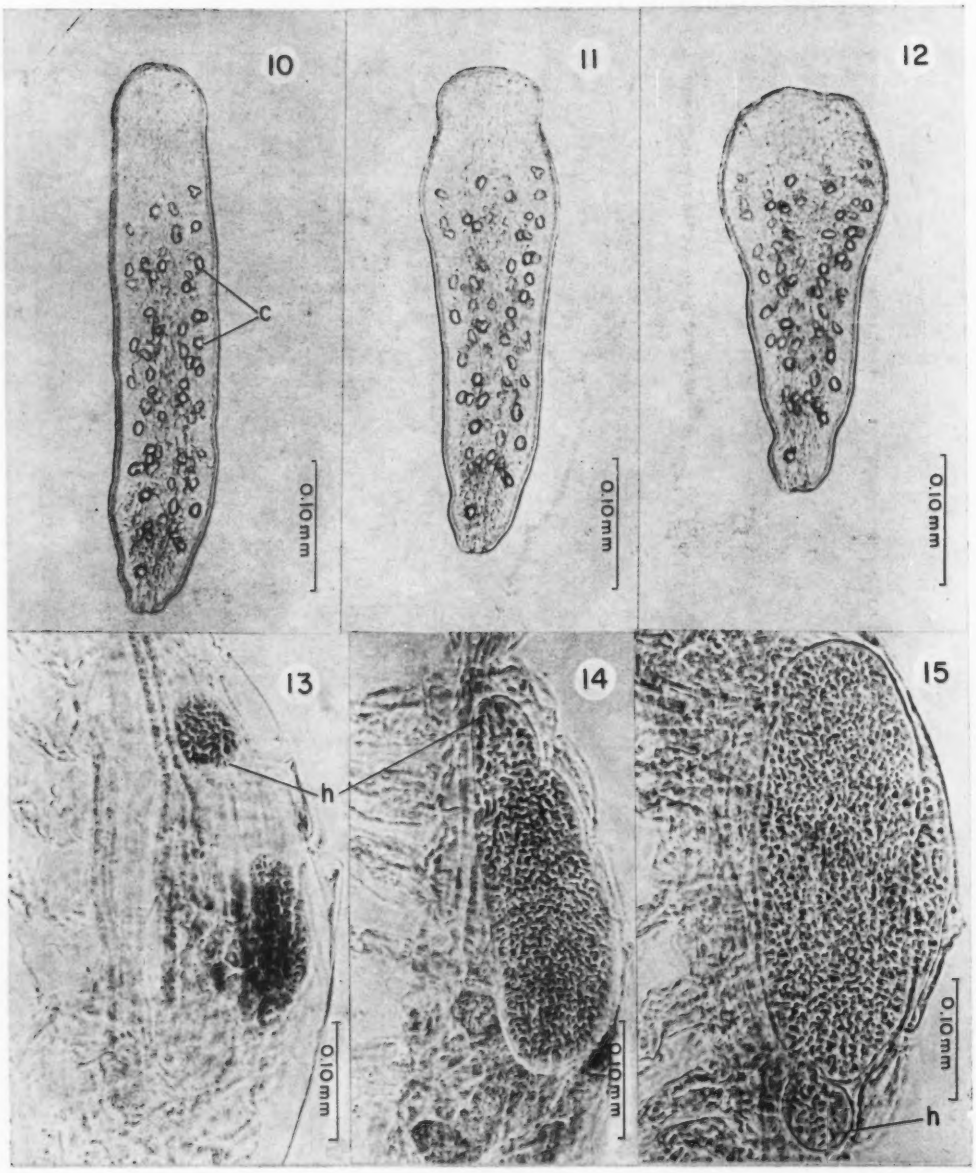

FIGURES 10–15.

FIG. 10, 11, 12. Infective metacestode in different stages of contraction (live, cercomer detached); FIG. 13. 20-day metacestode cultured at 5 C (permanent mount); FIG. 14. 20-day metacestode cultured at 10 C (permanent mount); FIG. 15. 20-day metacestode cultured at 15 C (permanent mount). h = hooks; c = calcareous corpuscles.

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at the end of 20 days. In copepods reared at 5 C the worms appeared little developed past the oncosphere stage (Fig. 13); worms in copepods reared at 10 C were large and elongate, and the formation of the cercomer was in progress (Fig. 14); in copepods reared at 15 C development of the metacestode was nearly complete and the cercomer was almost fully formed (Fig. 15).

Infection of the Final Host

Attempts to induce infection in sockeye fry were 39% successful, 70 of 179 fish fed infective metacestodes becoming infected. In order to assess the possible influence of certain factors on establishment of infection, comparisons were made within the following groups: 1, fry fed infected *C. scutifer* vs. fry fed infected *C. bicuspidatus*; 2, fry fed infected *Cyclops* directly vs. fry fed infected *Cyclops* via *Daphnia*; 3, fry of various length classes 26–35 mm through to 56–65 mm.

The above comparisons produced no evidence of an association between successful establishment of infection in fish and 1) choice of intermediate host species, or 2) manner of presentation of intermediate host. On the other hand, the results of experimental infection of fry of various lengths (Fig. 16) show a strong tendency for smaller fish to be more readily infected.

In the early stage of fish infection the worm, now an acaudate bothrio-plerocercoid, was located usually in the distal extremity of a pyloric caecum, and exceptionally in the intestine; residence of plerocercoids in the intestine was obviously temporary since advanced stages of the parasite were not found in this location.

Development in Final Host

Once established in the fish, the plerocercoid continues its development to the adult stage. The onset of segmentation, recognized approximately 30 days later in fish held at 10 C, is reflected in a series of 3–4 ill-defined transverse grooves (Fig. 8). No genitalia are discernible at this stage.

The small number of worms established experimentally in fish, all removed after 43 days or less, provide insufficient material for a complete account of the development of the adult worm to the egg-shedding stage. Examination of naturally infected fry shows that infections were acquired in Babine Lake as early as May 23, and the earliest occurrence of mature worms was in a sample taken on October 19 of the same year when out of 31 fingerlings, 12 of which were infected, six mature worms were recovered, one shedding eggs containing oncospheres.

Discussion

The results of this study agree essentially with the findings of Vik (1963) who reported briefly on the successful culturing of *E. salvelini* from egg to caudate bothrio-plerocercoid in the cyclopoid copepod *Cyclops strenuus*.

Observations in the laboratory suggest that in nature embryonated eggs are expelled into the fish's intestine, probably under pressure resulting from contortions of the worm, and eliminated with the feces. When the egg is ingested by the copepod, the capsule ruptures, releasing the coracidium into the gut where the embryophore is digested. While the results obtained with trypsin–versene digest suggest that enzymes play a part in this digestion, further investigation is required to determine precisely the role of enzymes. Voge and Graiwer (1964) described a trypsin–amylase "hatching solution" that they found to be effective in liberating oncospheres of *Hymenolepis diminuta*. It is likely that in the milieu of the copepod gut and its contents

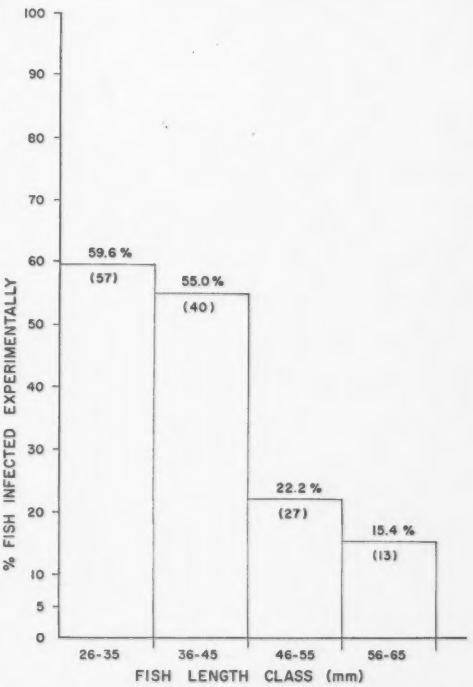

FIG. 16. *Oncorhynchus nerka* fry: susceptibility to experimental infection vs. size. (Numbers in parentheses indicate sample size.)

the highly active oncosphere is liberated mechanically even before digestion of the envelope is complete.

Several species of adult and near-adult *Cyclops* were readily infected, while other zooplankters tested proved consistently refractory. The observations summarized in Fig. 6 point to copepod size as a limiting factor, with smaller stages of *C. scutifer* and *C. bicuspidatus* unsuitable as hosts possibly because of an inability to ingest eggs. Other factors may limit infection in copepods of other genera; for example, oncosphere hooks found in the gut of *Diaptomus* sp. following exposure to eggs, suggest that ingestion and probably hatching of eggs did occur, but that the oncospheres did not pass to the haemocoel. In such copepods the oncosphere may have been defeated by an impenetrable gut wall, a destructive gut environment or both.

The level of natural infection found in *Cyclops* from Babine Lake may appear low, considering the incidence of infection observed in resident smolts. Awachie (1966) similarly observed a disproportionately low incidence of infection in the gammarid intermediate host of the trout cestode, *Cyathocephalus truncatus*. The information acquired in the course of this study so far does not provide a ready explanation of this suggested discrepancy; the following suggestions are speculative:

1. The feeding activity of fry is sufficiently intense that a relatively low incidence of copepod infection is still adequate to ensure the levels of infection observed in fish.

2. Certain aspects of the behavior of infected *Cyclops* lead to their being selectively preyed upon by fry.

3. Discrete foci of infection occur in Babine Lake and plankton sampling possibly occurred outside these zones.

4. The time of maximum prevalence of infected copepods is of a limited duration not coinciding with my sampling periods.

Results of this study indicate 30 days as the approximate time required for development from egg to infective metacestode at 10 C. Nevertheless, differences in rate of development have been observed among worms cultured under identical conditions of temperature, time, intensity of infection, and host species. These differences, similar to those noted by Mueller (1959) for the procercoid phase of *Spirometra mansonoides*, and apparently attributable to intrinsic differences among individual metacestodes, tend to make the effects of external influences difficult to assess. For instance, preliminary analysis of our material suggests that the "crowding effect," referred to by several workers, notably Read (1951), applies in *E. salvelini* development, in that in single infections in copepods worms grow better than they do in multiple infections. Similarly it appears that faster development occurs at higher temperatures. Assuming that the suggested influences of crowding, temperature and intrinsic variability are real in this case, and acting independently, assessment of the separate impact of each factor would require more data than the present study provides. Nevertheless, the clear-cut differences in size and development observed consistently among worms cultured at 5, 10, and 15 C (Fig. 13–15) leave no doubt that rate of development is directly related to temperature.

While the experimental results summarized in Fig. 16 indicate that smaller fry are more susceptible to infection, the reasons for this are as yet unclear. Two suggestions appear worth considering:

1. The small size and feeble walls of the pyloric caeca in the small fish permit a tight fit for the plerocercoid and enhance its ability to attach by bothria that are still poorly developed.

2. Immune systems exist in sockeye salmon that normally discourage establishment of *E. salvelini* and these systems are not yet fully developed in smaller fry. Kennedy and Walker (1969), McVicar and Fletcher (1970), and Kennedy (1971) suggested that immune systems operate against certain intestinal cestodes of fishes.

The occasional occurrence of plerocercoids in the intestine of fry suggests delayed digestion of the host copepods, resulting in release of the infective metacestodes at some distance beyond the pyloric region. Presumably such worms are ultimately voided with the feces or migrate to the pyloric caeca. Archer and Hopkins (1958), Hopkins (1959), and Bråten and Hopkins (1969) have described the tendency of immature *Diphyllobothrium*, *Proteocephalus* and *Hymenolepis*, respectively, to attach initially in the posterior regions of the intestine and later migrate anteriorly to their definitive sites.

The intensity of infection seen in smolts leaving Babine Lake seldom exceeds four adult worms, as opposed to intensities in excess of six worms often found in freshly infected fry. It remains to be determined whether this apparent reduction in intensity of infection is a manifestation of a worm population control mechanism, e.g. in response to crowding, or due to the death of the more severely infected fish.

While some worms attain maturity and may release eggs after only 5 months in the final host, i.e. as early as mid-October, the following evidence suggests that the shedding of eggs essential to the regeneration of *E. salvelini* in Babine Lake occurs primarily in the following spring.

1. Observation of larval worms cultured in vivo indicates that development is considerably depressed at 5 C, which exceeds the average lake temperature during most of the winter (D. M. Farmer unpublished data). If this influence of temperature applies as well to the worm in the final host, sexual development in *Eubothrium* could conceivably be inhibited at least during the period of ice cover and resumed about the time of spring thaw. Field observations tend to support this contention in that the first smolts trapped at the lake outlet in spring carry worms with mature but not gravid segments, whereas egg-shedding worms commonly occur 2–3 wk later.

2. Sockeye fry, the final link in the life cycle, do not enter the lake until May–June (McDonald 1969).

While terminal portions of worms in vitro appear to die, yet remain attached to the rest of the body, dead segments have not been encountered in worms freshly removed from fish; the observed explusion of portions of strobilae from fish, also noted by Smith (1973), suggests that as terminal portions of worm become exhausted of eggs they are detached and swept out by gut movements and the passage of feces. Wardle and McLeod (1952) indicated that the breaking away of strips of exhausted segments from the strobilae, termed "pseudapolysis," occurs commonly in the pseudophyllidean *Diphyllobothrium latum*.

Several questions raised in the course of this report would be best investigated by study of a large number of infected fish held captive from the initial stage of infection to the time of cestode egg-shedding. Regular examination of such fish through this period would provide data for a stepwise account of the development of the worm in the final host as regards location, growth, and maturation; controlled experiments could provide information regarding the response of the worm to crowding, diet, and temperature, as well as effects of infection on the vitality and survival of the host.

Acknowledgments

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Effect of Temperature and Antigen Dose on the Antibody Response of Juvenile Coho Salmon (*Oncorhynchus kisutch*) to *Aeromonas salmonicida* Endotoxin[1]

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PATERSON, W. D., AND J. L. FRYER. 1974. Effect of temperature and antigen dose on the antibody response of juvenile coho salmon (*Oncorhynchus kisutch*) to *Aeromonas salmonicida* endotoxin. J. Fish. Res. Board Can. 31: 1743–1749.

Specific antibody production in response to an intraperitoneal injection of *A. salmonicida* endotoxin first occurred in juvenile coho salmon (*Oncorhynchus kisutch*) held at 6.7, 12.2, and 17.8 C at 4, 2 and 1 wk, respectively. *Aeromonas salmonicida* endotoxin was antigenic to juvenile coho salmon at all doses between 1 and 5000 μg administered intraperitoneally with and without an aluminum hydroxide carrier.

The endotoxin was nontoxic to juvenile coho salmon when 1–5000 μg was injected intraperitoneally and the fish held at temperatures from 6.7 to 17.8 C or when "free endotoxin" was administered by the intramuscular or intraperitoneal route. *Aeromonas salmonicida* endotoxin was toxic to mice, however.

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La production d'anticorps spécifiques en réponse à une injection intrapéritonéale d'endotoxine d'*A. salmonicida*, chez des jeunes saumons coho maintenus à 6.7, 12.2 et 17.8 C, apparaît pour la première fois à 4, 2 et 1 semaines respectivement. L'endotoxine d'*Aeromonas salmonicida*, à tous les dosages compris entre 1 et 5000 μg administrés par voie intrapéritonéale, avec ou sans hydroxyde d'aluminium comme porteur, cause la production d'antigènes chez les jeunes saumons coho.

L'endotoxine est non toxique pour les jeunes saumons coho lorsqu'une dose de 1 à 5000 μg est injectée par voie intrapéritonéale et que les poissons sont maintenus à des températures de 6.7 à 17.8 C, ou encore quand «l'endotoxine libre» est administrée par voie intramusculaire ou intrapéritonéale. Par contre, l'endotoxine d'*Aeromonas salmonicida* est toxique pour les souris.

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THE temperature effect on antibody production in fish has been reviewed (Smith 1940; Snieszko and Friddle 1949; and Ridgeway et al. 1966). In contrast to homeothermic vertebrates, the immune response of poikilothermic vertebrates is influenced and even controlled by the environmental temperature of the animal. To be useful in a hatchery situation it is essential that the immunization program be carried out early in spring before a rise in water temperature produces losses due to increased disease. Therefore, a precise understanding of the effect of water temperature on the immune response of each species of fish is important.

In characterizing the immune response of these animals it is also important to gain some knowledge of the influence dose has on the level of response.

This paper describes the effects of temperature and antigen dosage on the antibody response of juvenile coho salmon to *Aeromonas salmonicida* endotoxin.

Materials and Methods

EXPERIMENTAL ANIMALS

Juvenile coho salmon (*Oncorhynchus kisutch*) obtained from the Fish Commission of Oregon (FCO) were held in 83.3-liter (22-gal) or 681-liter (122-gal)

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fiberglass tanks supplied with 12.2 C well water at a rate of 1.9 liters/min (0.5 gal/min) and 7.6 liters/min (2 gal/min), respectively, and fed Oregon Moist Pellets (Hublou 1963). Ambient water temperature was 12.2 C. Temperature control equipment was used to conduct experiments at temperatures from 6.7 to 23.3 C.

Endotoxin Preparation

The *Aeromonas salmonicida* cultures used in this work were isolated from coho salmon at FCO Siletz River Hatchery in 1967 and chinook salmon (*O. tshawytscha*) at FCO South Santiam Hatchery in 1970. These strains are designated As Sil 67 and As SS-70, respectively.

For endotoxin extraction, bacterial cells were grown at 20 or 23 C in 20–30-liter lots of Trypticase Soy Broth (TSB) within a Fermacell Fermentor, Model CF 5 (New Brunswick Scientific Co., New Brunswick, New Jersey). To replenish carbohydrate supply in the medium, a concentrated filter sterilized (0.45 nm) dextrose solution (500 ml 28% dextrose→1000 ml 60% dextrose) was added during incubation. The culcure was monitored periodically for purity, pH, and tell density by aseptically withdrawing samples from the culture vessel. The fermentor culture was collected and cells harvested in a Sharples Super Centrifuge Type TIP. Cells were then washed twice in 0.85% saline. The maximum yield of cells was obtained by addition of dextrose after medium carbohydrates had been depleted. This condition was indicated by a rise in pH resulting from amino acid metabolism.

For endotoxin extraction, the wet cells were suspended in distilled water at a level of 200 mg cells/ml H_2O. The endotoxin was extracted by the method of Staub (1967). Nitrogen content of endotoxins was determined by the Nessler reaction after Kjeldahl digestion (Williams and Chase 1967). Dry weight of the As Sil 67 endotoxin was obtained after drying to constant weight in vacuo at 63 C over silica gel.

An aluminum hydroxide adsorbant was prepared by the procedure of Rethy (1965) and added to the *A.*

Table 1. Design of experiments to determine the effect of dosage and temperature on the antibody response of juvenile coho salmon (*Oncorhynchus kisutch*) to *Aeromonas salmonicida* endotoxin.

	Experiment		
	A	B	C
Parameter	Effect of dose administered	Response to *Aeromonas salmonicida* culture supernatant	Effect of temperature on antibody response
Temp	12.2 C	12.2 C	6.7, 12.2, 17.8 C
Total injected dose	1–5000 μg *A. salmonicida* endotoxin with or without $Al(OH)_3$	0.1 ml	1000 μg *A. salmonicida* endotoxin with $Al(OH)_3$
Injection schedule	two 0.1-ml ip injections four weeks apart	one 0.1-ml ip or im injection	one 0.1-ml ip injection
Monitor	toxicity–death; antibody response-pooled serum (20 fish) and individual serum samples from another 10 fish from each treatment taken 8 and 12 wk after first injection	toxicity–death; antibody response — individual serum samples from five fish 22 days after injection	antibody response — individual serum samples from 10 (for 22.6-g fish) and 5 (for 6.5-g fish) animals at weekly intervals
Control injection	water or $Al(OH)_3$ in water	uninjected controls	$Al(OH)_3$ in water
Positive response	precipitin band or agglutinin titer equal to or greater than 160	agglutinin titer greater than 80	agglutinin titer equal to or greater than 160 for the 6.5-g fish and 320 for the 22.6-g fish
Mean animal weight	7 g	25.2 g	22.6, 6.5 g

salmonicida endotoxin at a level of 390 μg $Al(OH)_3$/1000 μg endotoxin. The endotoxin was administered to fish with and without this carrier.

Injection and Bleeding Procedures

Fish were injected with *A. salmonicida* endotoxin by both the intraperitoneal (ip) and the intramuscular (im) route.

Juvenile coho salmon blood was obtained and treated as reported previously (Paterson and Fryer 1974) to obtain serum. The experimental design of the vaccination experiments is outlined in Table 1.

Serum Monitoring

The microtiter agglutination and gel precipitin procedures used to monitor the antibody response of juvenile coho salmon have been described previously (Paterson and Fryer 1974) and data obtained were treated in a similar manner to determine the percent response.

Toxicity Determinations

Fish were administered *A. salmonicida* endotoxin doses up to 2500 μg/ip injection. The effect of *A. salmonicida* endotoxin on warm-blooded animals was investigated using mice as representative animals. LD_{50} determinations were carried out using 0.5-ml ip injections, a dilution interval of 0.5 $\log_{10}$, and a sample size of five mice per dilution. LD_{50} values of this endotoxin in the mice were determined by the method of moving averages (Meynell and Meynell 1965).

Mice were observed for signs of a toxic reaction after injection and on death were examined for gross external and internal pathology. All deaths contributed to the LD_{50} values. Control injections carried out with LD_{50} determinations consisted of 0.85% saline for mice.

An *A. salmonicida* culture supernatant designated "free endotoxin" was prepared and injected to observe its toxic and antigenic effects on juvenile coho salmon. Crutchley et al. (1967) previously demonstrated that "free endotoxins" are liberated into the culture supernatants of a wide variety of Gram negative organisms. Before injecting, the broth culture of *A. salmonicida* was centrifuged to remove cellular material and sterilized by filtration (0.45 nm).

Results

Endotoxin Extraction

A total of 8.6 g of lyophilized *A. salmonicida* endotoxin was extracted from 864 g of wet packed cells, an average yield of 1% of wet weight of bacteria. The *A. salmonicida* As Sil 67 endotoxin was analyzed as 6.6% water and 2.46% nitrogen by weight.

Table 2. Antibody response of juvenile coho salmon (*O. kisutch*) (fish held at 12.2 C) to various levels of *A. salmonicida* endotoxin administered intraperitoneally with and without $Al(OH)_3$ carrier.

	Pooled samples[a]		Individual samples[b]	
	Agglutinating antibody titer		Mean agglutinating antibody titer	
Dose of endotoxin per fish	8-wk samples	12-wk samples	8-wk samples	12-wk samples
0	120	160	33	60
1 μg	640	480	336	324
10 μg	960	1280	488	256
100 μg	960	640	288	496
1000 μg	1280	640	360	1093
5000 μg	1280	640	204	296
$Al(OH)_3$	160	80	36	32
1 μg + $Al(OH)_3$	960	640	1426	92
10 μg + $Al(OH)_3$	1720	1280	980	302
100 μg + $Al(OH)_3$	1720	960	3376	720
1000 μg + $Al(OH)_3$	2560	960	1468	164
4000 μg + $Al(OH)_3$	1280	960	1228	548

[a] 20 fish/pooled sample.
[b] 10 fish/group. These are separate animals from those used in the pooled sample.

Antibody Response to Endotoxin

In the first sample taken 8 wk after the first injection, elevated antibody titers were observed in fish injected with all endotoxin levels, ranging from 1 to 5000 μg (Table 2). Although the response appears greater with the aluminum hydroxide carrier, the difference is not statistically significant except in the case of the 100-μg dose. The average percent response for the two sampling periods ranged from 55 to 69% and the percent response of each group at the two sampling periods (Table 3) indicated that not all animals responded to administration of this antigen. An antibody response was also observed in all coho salmon receiving the "free endotoxin" by both the ip and the im route (Table 4).

Endotoxin Toxicity

Aeromonas salmonicida endotoxin was nontoxic to juvenile coho salmon under all conditions of dosage and temperature. No mortalities resulted from the ip injections of *A. salmonicida* endotoxin into juvenile coho salmon held at 12.2 C (Table 1). This apparent lack of toxicity to fish has previously been reported (Berczi et al. 1966; Wedemeyer et al. 1969). *Aeromonas salmonicida* "free endotoxin" procured by a very mild procedure was also not toxic to juvenile coho salmon.

Table 3. Percent antibody response[a] of juvenile coho salmon[b] 8 and 12 weeks after two injections of *A. salmonicida* endotoxin.

Aeromonas salmonicida endotoxin	% antibody response[c]	
Dose/fish	8 wk after first injection	12 wk after first injection
0	0	10
1 μg	50	70
10 μg	60	60
100 μg	80	60
1000 μg	80	80
5000 μg	60	50
Average	66	64
$Al(OH)_3$	0	0
1 μg + $Al(OH)_3$	60	20
10 μg + $Al(OH)_3$	90	45
100 μg + $Al(OH)_3$	80	50
1000 μg + $Al(OH)_3$	80	30
4000 μg + $Al(OH)_3$	50	90
Avg	72	47
Overall avg of antigen-injected fish	69	55

[a]Percentage of 10 fish samples with agglutinating antibody titer equal to or greater than 160.
[b]Holding temperature = 12.2 C.
[c]Mean agglutinating antibody titer of control animals with 95% confidence limits (CL), 8-wk samples = 26 (4–176), 12-wk samples = 39 (12–122).

Table 4. Antibody response of juvenile coho salmon to an *A. salmonicida* "free endotoxin" (a culture supernatant solution).[a]

Antigen administration	Agglutinating antibody titers
Intramuscular injection of "free endotoxin"	5120, 5120, 640, 320, 320
Intraperitoneal injection of "free endotoxin"	1280, 1280, 640, 640, 320
Controls (no injection)	80, 80, 40, 40, 40

[a]Individual serum samples taken from five fish in each treatment 22 days after injection.

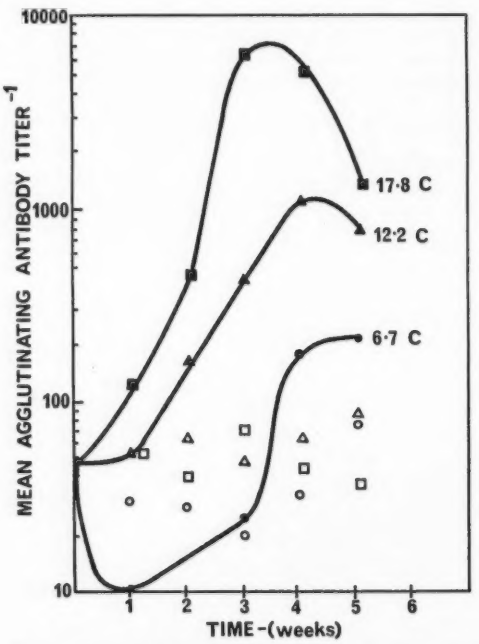

Fig. 1. Antibody response of 6.5-g juvenile coho salmon (*Oncorhynchus kisutch*) held at 6.7, 12.2, and 17.8 C to a single intraperitoneal injection of *Aeromonas salmonicida* endotoxin. Each point represents the mean of the reciprocal agglutinating titer for a 5-fish sample. ■, ▲, ●, = antigen-injected animals; □, △, ○, = control animals injected with $Al(OH)_3$ only.

The comparative toxicity of *A. salmonicida* endotoxin to salmon and mice suggests that this is a typical endotoxin.

Animal	LD_{50} (*mg* endotoxin/*kg* animal)	Mean animal wt (*g*)
Coho salmon	>714	7
Mice	21.6	24.8

In contrast to results obtained with coho salmon, the *A. salmonicida* endotoxin was lethal to mice. Mice injected with *A. salmonicida* endotoxin showed reduced activity 2 h after injection and by 10 h post injection they continued to show this symptom, plus a waddling gait and closed eyes. Survivors after 24 h were inactive and exhibited ruffled hair and closed eyes. Internal examination revealed occasional swollen intestines and hemorrhaged spots on the lungs. Symptoms similar to these have been reported for a toxic portion of *Pseudomonas aeruginosa* (Meinke and Berk 1970).

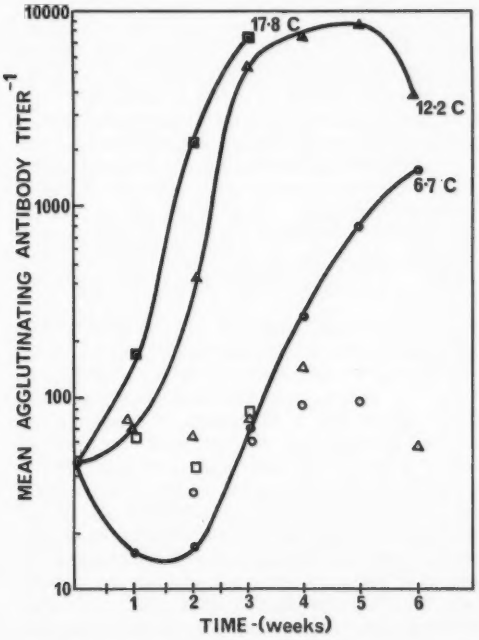

FIG. 2. Antibody response of 22.6-g juvenile coho salmon held at 6.7, 12.2, and 17.8 C to a single intraperitoneal injection of *A. salmonicida* endotoxin. Each point represents the mean of the reciprocal agglutinating titer for a 10-fish sample. ■, ▲, ●, = antigen-injected fish; □, △, ○ = control animals injected with $Al(OH)_3$ only.

EFFECT OF TEMPERATURE ON THE ANTIBODY RESPONSE

The dynamics of the coho salmon antibody response at 6.7, 12.2, and 17.8 C indicates the significant immunological competence that these animals exhibit at a variety of temperatures (Fig. 1 and 2). The proportion of animals with an elevated antibody response (Table 5) emphasizes that the temperature affects mainly the time taken to respond. Results from separate experiments utilizing different sized animals indicates comparable responses at each temperature.

At 6.7 C, an antibody response was first observed in both sizes of fish 4 wk after the antigen injection, but all animals did not respond at this time. Prior to the initial response at 6.7 C agglutinating antibody levels of fish injected with the antigen had decreased below that of the control fish. In these animals, antibody levels normally present in control fish had been neutralized by injected antigen leaving titers of <10.

TABLE 5. Percent antibody response of juvenile coho salmon[a] to *A. salmonicida* endotoxin[b] at three temperatures.

Time after injection (*wk*)	% response[c] at various temp (*C*)					
	Experiment 1[d]			Experiment 2[e]		
	6.7	12.2	17.8	6.7	12.2	17.8
0	0	0	0	0	0	0
1	0	0	20	0	0	60
2	0	40	100	0	60	80
3	0	90	100	0	80	100
4	40	100	–[f]	20	100	80
5	100	100	–[f]	60	80	60
6	80	80	–[f]	–[f]	–[f]	–[f]

[a]Mean fish weight Experiment 1: 22.6 g, Experiment 2: 6.5 g.

[b]Each animal injected ip with 1000 μg of *A. salmonicida* endotoxin in $Al(OH)_3$.

[c]Mean agglutinating antibody titers of control animals with 95% CL were as follows:

Experiment 1: 6.7 C = 51 (12–221), 12.2 C = 66 (21–208), 17.8 C = 52 (17–157).

Experiment 2: 6.7 C = 33 (9–119), 12.2 C = 55 (27–112), 17.8 C = 45 (20–103).

[d]Experiment 1: 10 fish/sample

[e]Experiment 2: five fish/sample

[f]Not done.

At 12.2 C, a response was detected 2 wk after antigen administration. The response at the 2-wk period was detected in only 50% of the animals tested. Maximum antibody levels appeared 4-5 wk after injection and the highest antibody levels were obtained in the larger 22.6-g fish.

At 17.8 C, an antibody response could be detected as early as 1 wk after injection. In the experiment with fish weighing 22.6 g each, only two of the 10 animals had responded by the end of 1 wk. However, three of five of the smaller fish (6.5 g/fish) had responded by this time. Maximum titers were observed in the smaller fish 3 wk after injection.

Experiments with the larger 22.6-g animals were carried out for 15 wk after injection. At both 6.7 and 12.2 C high levels of antibody were maintained through the samples taken 13 wk after injection. Antibody levels and percent response of animals had markedly declined by the 15-wk sampling period.

	% response at	
Time after injection (*wk*)	6.7 C	12.2 C
0	0	0
5	100	100
13	100	80
15	50	40

Discussion and Conclusions

Endotoxin from *A. salmonicida* provides an excellent antigenic system for studying the immune response of fish. This antigen was injected into fish and elicited a good immune response without using Freund's complete adjuvant. This endotoxin was also administered quantitatively and elicited a strong antibody response which can be monitored in vitro by means of both agglutination and precipitin reactions. The high degree of antigenicity of *A. salmonicida* endotoxin for fish is indicated by the positive immune response obtained after injection of only 1 μg.

At 6.7, 12.2, and 17.8 C, juvenile coho salmon synthesized anti-*A. salmonicida* antibodies in 4, 2, and 1 wk after injection, respectively. This more rapid antibody production at higher temperatures is characteristic of poikilothermic animals. The rate of antibody synthesis and time of detection after antigen administration to coho salmon are comparable to values reported for eels, trout, and goldfish (Muroga and Egusa 1969; Chiller et al. 1969; Trump and Hildemann 1970).

At 6.7 C a marked decrease in antibody level was observed for the first 2 wk after injection. This is a reflection on the efficiency of an intraperitoneal injection of this endotoxin which circulated throughout the animal and probably complexed with serum antibody to remove the antibody as such from the humoral system.

In all but the smallest fish tested, control animals possessed antibody titers ranging from 10 to 160. Similar observations have been made previously (Bisset 1948; Frost 1968; Krantz and Heist 1970). Bisset (1948) demonstrated agglutinin titers in the sera of perch against bacteria isolated from the peritoneal and opercular cavities of these animals. Frost (1968) and Krantz and Heist (1970) detected anti-*A. salmonicida* antibodies in nonimmunized trout and salmon.

Several possible explanations for the presence of antibody levels in these control animals could be made. Krantz and Heist (1963) suggested that contact with *A. salmonicida* in the environment gives rise to this background level of antibodies. In addition, the anti-*A. salmonicida* antibodies produced by coho salmon are extremely efficient in agglutinating these bacteria; only 0.006 μg antibody N/ml serum is necessary to detect the presence of antibody with these bacteria (Cisar and Fryer 1974).

Endotoxins as represented by *A. salmonicida* endotoxin are nontoxic to juvenile coho salmon in the tolerable temperature range of these animals. This lack of toxicity to fish has previously been reported (Berczi et al. 1966; Wedemeyer et al. 1969). The LD_{50} values obtained by the administration of *A. salmonicida* endotoxin to mice shows that the toxicity of this endotoxin is comparable to endotoxins of *Escherichia coli* for the same animals as reported by Berczi et al. (1966). *Aeromonas salmonicida* endotoxins are also quite toxic to chick embryos (A. J. Ross, Seattle, Wash., personal communication).

The experiments described in this paper have documented some of the immunological capabilities of juvenile coho salmon and provided a confidence factor for future immunization programs. This work showed that a serological response can be obtained by these fish at a variety of temperatures from 6.7 to 17.8 C. These and other data demonstrate that hatchery stocks of juvenile coho salmon can be effectively immunized (Paterson and Fryer 1974) at hatcheries even if low water temperatures prevail. With the assurance that these animals are capable of being immunized under hatchery conditions, investigation should be focused on other problems such as method of vaccine preparation and application methods.

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Immune Response of Juvenile Coho Salmon (*Oncorhynchus kisutch*) to *Aeromonas salmonicida* Cells Administered Intraperitoneally in Freund's Complete Adjuvant[1]

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The antibody response of a population of juvenile coho salmon (*Oncorhynchus kisutch*) held at 12 C approached 100% following a single parenteral vaccination with *Aeromonas salmonicida* cells emulsified in Freund's complete adjuvant. This response indicates that coho salmon behave as a homogeneous population with respect to immunological competence.

Fish as small as 1.2 g produced antibodies as early as 4 wk after administration of this vaccine; high levels of antibody persisted for at least 1 yr after injection. This single vaccination provided juvenile coho salmon with protective immunity against furunculosis.

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La production d'anticorps chez une population de jeunes saumons coho (*Oncorhynchus kisutch*) maintenus à 12 C se rapproche de 100% après une seule vaccination des parents avec des cellules d'*Aeromonas salmonicida* émulsifiées dans le «complete adjuvant» de Freund. Cette réponse indique que, sous le rapport de la suffisance immunologique, le saumon coho se comporte comme une population homogène.

Des poissons ne pesant que 1.2 g produisent des anticorps dès 4 semaines après l'administration du vaccin; les anticorps persistent à des niveaux élevés pendant au moins 1 an après l'injection. Cette unique vaccination fournit aux jeunes saumons coho une immunité contre la furonculose.

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ALTHOUGH the immune responses of fish and other poikilothermic animals has been poorly understood in the past, work concentrated on the area of fish immunity during the last decade (reviewed by Klontz and Anderson 1970; Snieszko 1970) has clarified the picture considerably. Investigations of the immune responses of certain trout and salmon species to *Aeromonas salmonicida* demonstrated the production of protective antibodies in brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*) following injection of *A. salmonicida* in an oil adjuvant (Krantz et al. 1963) and the protective nature of rainbow trout (*Salmo gairdneri*) anti-*A. salmonicida* serum through passive immunization of juvenile coho salmon (Spence et al. 1965). Further research is now necessary for the application of this knowledge as is currently done for homeothermic animals. Information is now required on various parameters affecting the immune response of fish such as animal age, antigen preparation, dosage, route of administration, effect of temperature, percent response by a population, and the protection offered. Accordingly, this paper describes the antibody response of juvenile coho salmon at 12.2 C to a single intraperitoneal injection of *A. salmonicida* cells emulsified in Freund's complete adjuvant. The parameters investigated included the antibody response of a population, duration of response, immunological competence of juvenile animals, and the protective nature of this vaccination procedure.

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Materials and Methods

Experimental Animals

Juvenile coho salmon (*Oncorhynchus kisutch*) obtained from hatcheries of the Fish Commission of Oregon (FCO) were held in 681-liter (180-gal) or 83.3-liter (22-gal) fiberglass tanks supplied with 12.2 C well water at a rate of 7.6 liters/min (2 gal/min) and 1.9 liters/min (0.5 gal/min), respectively, and fed Oregon Moist Pellets (Hublou 1963) for the duration of the studies.

Bacterial Cultures

The *A. salmonicida* cultures used were designated As Sil 67 (isolated from coho salmon at FCO Siletz River Salmon Hatchery in 1967) and As SS70 (isolated from chinook salmon at FCO South Santiam Salmon Hatchery in 1970).

To maintain antigenic uniformity, cellular antigen preparations for use in immunization and agglutination procedures were produced from lyophilized stocks. The *A. salmonicida* cells were cultured on Furunculosis agar (FA) (Spence et al. 1965) for 24 h at 20 C, then harvested and washed 3 times in cold physiological saline. For immunization, formalin was added to a final concentration of 0.4%. Cells for use in agglutination tests were not formalin killed.

A virulent *A. salmonicida* culture for challenging vaccinated animals was prepared from lyophilized furunculosis-infected salmon kidney. Before use as a challenge, the infected kidney was suspended in Brain Heart Infusion (BHI) broth and then cultured on BHI agar for 24 h at 18 C. Cells were harvested, washed 3 times in phosphate buffered saline, and then suspended in 0.85% saline.

Vaccination

Suspensions of formalin killed *A. salmonicida* were mixed 1:1 with Freund's complete adjuvant (FCA) and emulsified in a glass syringe or blender. The bacterial cell-adjuvant emulsions were injected intraperitoneally (ip) just anterior to the pelvic fins with a 20 gauge needle.

Monitoring Procedures

Blood was obtained from the caudal vein of each juvenile coho salmon after the tail was severed just posterior to the adipose fin. The blood was allowed to clot for 1 h at room temperature followed by 12 h at 4 C after which the serum was separated from the blood cells by centrifugation at 2000 rpm.

All individual coho serum samples were analyzed for agglutinating antibody titer using a microtiter apparatus with disposable "V" bottom plates (Cooke Engineering Co., Alexandria, Virginia). Twofold serial dilutions were made in 0.85% saline from initial 1:5 or 1:10 serum dilutions. After serial serum dilutions were made, 25 or 50 μl of fresh *A. salmonicida* antigen preparation previously adjusted to 0.9 OD at 520 nm was added to each well to give final maximum dilutions of 20,480 or 40,960. The plates were gently mixed, covered, and incubated for 3 h at room temperature, followed by 12 h at 4 C after which the results were recorded. A positive agglutination reaction was shown by the uniform dispersal of bacterial cells over the bottom of the microtiter well. A negative agglutination reaction was indicated by the presence of a small button of bacterial cells at the very bottom of the V-shaped microtiter plate well.

The agglutinin titers were reported as the reciprocal of the maximum dilution showing the positive agglutination reaction and arithmetic means of the reciprocal values were calculated for data shown in the tables. Reciprocal titer values were converted to logarithmic values to the base 10 for statistical analysis and construction of 95% confidence limits (CL) around the control means. Titers greater than the 95% CL for the control animals constituted a positive response.

Agar gel precipitin tests were conducted on 25- × 75-mm slides according to the method of Campbell et al. (1970) modified as described below. Wells 3 mm in diameter and spaced 4 mm apart were each filled with 0.01 ml of antigen or undiluted serum. A 1×10^4 μg/ml stock of *A. salmonicida* endotoxin (Paterson and Fryer 1974) was used in each antigen well for routine monitoring of anti-*A. salmonicida* precipitins present in serum.

Experimental Design

To observe the antibody response of groups of individual fish, as a function of time, juvenile coho salmon (mean weight 20 g/fish) were obtained from the FCO Alsea Salmon Hatchery. Two groups of 1500 fish were each placed in a 681-liter, 1.22-m (180-gal, 4-ft) circular tank with a flow rate of 7.6 liters/min (2 gal/min). Each fish of one group was injected ip with 1.8×10^9 *A. salmonicida* (As Sil 67) cells emulsified in FCA. The second group of fish, designated as a control, was injected ip with 0.1 ml of saline emulsified in FCA. The immune response was monitored by bleeding 25 animals from each group every 2 wk, beginning on the date of injection but prior to injection. Individual serum samples were analyzed in duplicate for agglutinating antibody titer (to a maximum of 40,960) using the microtiter technique. Specific precipitating capacity of individual serum samples was examined on the micro gel diffusion slides.

To examine the immunological competence and the duration of the immune response of young animals held at 12.2 C, small juvenile coho salmon (mean weight 1.2 g/fish) were each injected ip with a volume of 0.025 ml containing 2.3×10^9 *A. salmonicida* (As Sil 67) cells emulsified in FCA. Uninjected animals were used as controls. Individual blood samples were taken from each of 5 or 10 vaccinated fish at about monthly intervals for a year after injection and the serum analyzed for agglutinating antibody titer (to a maximum of 20,480). Blood samples were taken and analyzed as above for control animals at 0, 43, 77, 106, and 140 days, respectively.

The degree of protection against a virulent *A. salmonicida* isolate (AS SS70) was also investigated. Juvenile coho salmon (mean weight 2.8 g/fish) held at 12.2 C were each administered an ip injection containing 1.2×10^9 formalin killed *A. salmonicida* (As SS) cells emulsified in FCA and then challenged 3 mo later with a single 0.1-ml ip injection of the virulent *A. salmonicida* strain (As SS70) cells. Control animals were injected with 0.85% saline emulsified in FCA and challenged as described above for vaccinated fish. Fish dying within 1 wk of injection were examined for the presence of *A. salmonicida*. Significance of protection offered by this vaccination procedure was tested using Chi square analysis.

Results

Population Response with Time

The antibody response following a single injection of antigen was first observed 4 wk after administration. Means of agglutinin titers from each 25-fish sample were extremely high, ranging from 10,860 to 28, 224 and these titers persisted at these levels from the fourth week to the last sampling at 14 wk (Table 1).

The control fish possessed antibody specific for *A. salmonicida* at titers ranging from 20 to 160. Starting 4 wk after the injection, between 88 and 100% of the vaccinated animals exhibited elevated antibody levels for the six sampling periods. The overall mean response of 96.6% indicates that juvenile coho salmon consist of a relatively homogeneous population with respect to immunological competence to *A. salmonicida* cellular antigens administered with Freund's complete adjuvant.

The detection of the juvenile coho immune response using the gel precipitin procedure lacked the sensitivity of the agglutination tests. Positive precipitin reactions were not detected unless the serum agglutinin titer was 1280 or greater.

Duration of Immunity and Immunological Competence of Small Fish

Serum samples were taken at monthly intervals from small fish injected with *A. salmonicida* cells emulsified in FCA. High agglutinating antibody titers were detected throughout the entire sampling period of 1 yr (Table 2). From the samples taken 77 days after injection and thereafter, virtually 100% of the population tested possessed elevated agglutinating antibody titers against *A. salmonicida*. This shows that animals as small as 1.2 g can be vaccinated successfully and that their immune state persists for at least 1 yr after injection.

Protective Immunity

The protective nature of a single vaccination is shown in Table 3. In the two trials, there were significantly more mortalities in the control than in the immunized group when data was analyzed. This demonstrates that protective immunity was conferred by the FCA-cell vaccination procedure.

Table 1. Antibody response[a] of juvenile coho salmon[b] (*Oncorhynchus kisutch*) to a single intraperitoneal injection of *Aeromonas salmonicida* cells emulsified in Freund's complete adjuvant.

Time after injection (*wk*)	Mean agglutinating antibody titer	Range of antibody titers	%[c] antibody response of the population[d]	Presence of precipitating antibody (No. positive/25 fish)
0	42	<20 to 80	0	0
2	38	20 to 80	0	0
4	10860	160 to ≧40960	96	16
6	21630	320 to ≧40960	100	23
8	23000	160 to ≧40960	96	16
10	26265	80 to ≧40960	88	24
12	28224	320 to ≧40960	100	22
14	27706	320 to ≧40960	100	23

[a]Temperature = 12.2 C.
[b]Mean fish weight = 20 g/fish.
[c]Overall mean agglutinating titer of control animals with 95% confidence limits = 52 (17.6–155). Titers greater than 160 indicated a positive response.
[d]Agglutinating titer measured in individual sera of 25 fish.

TABLE 2. Longevity of antibody response in juvenile coho salmon[a] injected intraperitoneally with *A. salmonicida* cells in Freund's complete adjuvant and held at 12.2 C.

Vaccinated animals time after injection (*days*)	No. of fish bled	Mean agglutinating antibody titer	Range of antibody titers	% antibody response[b] of vaccinated animals
0	10	22		0
43	10	562	20 to 1280	50
77	10	14624	2560 to ≥ 20480	100
106	5	20480	all ≥ 20480	100
140	5	20480	all ≥ 20480	100
219	5	20480	all ≥ 20490	100
261	5	16384	10240 to ≥ 20480	100
281	5	18432	10240 to ≥ 20480	100
324	5	20480	all ≥ 20480	100
356	5	16896	2560 to ≥ 20480	100
388	6	14912	320 to ≥ 20480	100

[a]Mean weight at $T_o = 1.2$ g.
[b]Mean agglutinating titer of uninjected control animals with 95% confidence limits = 21 (range 12–36). Titers greater than 40 constituted a positive response.

Discussion

Population Response with Time

The antibody response observed in coho salmon following injection of a cellular *A. salmonicida* antigen in FCA was detected later than a response obtained by injection of a soluble antigen (*A. salmonicida* endotoxin). With an FCA–*A. salmonicida* cell injection, the first elevated antibody titers were detected 4 wk post injection in animals held at 12.2 C. In contrast, the first elevated antibody levels were observed only 2 wk after injection with endotoxin at this temperature (Paterson and Fryer 1974).

This delayed response following an antigen–FCA injection has also been observed by Post (1963). One possible explanation for this phenomenon is the slow release of antigen when emulsified in adjuvant prior to injection. This slow release would allow the antigen to complex with serum antibody as it is produced, giving a false negative response. The soluble antigen (*A. salmonicida* endotoxin), in contrast, is neutralized and removed more rapidly from the circulatory system. Thus, the antibodies are detectable earlier in response to this soluble antigenic stimulation (Paterson and Fryer 1974).

The high antibody titer obtained with injection of *A. salmonicida* cells emulsified in FCA persists in fish for at least a year after injection. In comparison, titers obtained by injection of endotoxin with only an aluminum hydroxide carrier begin to decrease 5–6 wk after injection. The higher titers and longer duration of antibody response using FCA result from its ability to persist in the animals for long periods of time. Similarly, Krantz et al. (1963) reported that antibody titers from 640 to 10,240 in brown trout (*S. trutta*) persisted for 2-yr after administration of vaccine emulsified in an oil

TABLE 3. Challenge of vaccinated[a] and untreated[b] juvenile coho salmon with an intraperitoneal injection of cultured *A. salmonicida* (South Santiam) cells.

Sample size/group (No. of fish)	No. of viable cells injected	% mortality due to furunculosis		Chi square value
		Control	Immune	
15	1470	40	0	10.18*
20	2700	70	15	5.2**

[a]Injected ip with formalin killed *A. salmonicida* (South Santiam) cells emulsified in Freund's (complete) adjuvant.
[b]Injected ip with saline emulsified in Freund's (complete) adjuvant.
*Significant at 0.01 level. **Significant at 0.05 level.

adjuvant, but administration of vaccine without adjuvant produced an antibody response which returned to the preimmunization levels 3 mo after reaching peak titer.

With respect to immunological competence, the group of juvenile coho salmon injected with *A. salmonicida* cells plus FCA behaved as a very homogeneous population with an overall immune response of 96.6%. Gee and Smith (1941) and Hildemann (1962) reported on the homogeneity of immune response of carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*), respectively. This type of population response is critical if immunization programs are to be successful.

Data obtained from analysis of individual serum samples would allow a worker to project the percent potential immunological competence of large fish populations and aid in designing and performing immunization experiments of a parenteral or oral nature.

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Influence of Fenitrothion on Swimming Velocities of Brook Trout (*Salvelinus fontinalis*)

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PETERSON, R. H. 1974. Influence of fenitrothion on swimming velocities of brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 31: 1757–1762.

The critical swimming velocities of brook trout (*Salvelinus fontinalis*) following exposure to 150, 500, and 1500 μg/liter fenitrothion were 100, 83, and 70%, respectively, of the performance of control fish. This represented 35 and 60% decrease in power output, respectively, for the two higher concentrations.

At step-test intervals longer than 15 min, the critical velocity (ca 4.5–5 lengths [L]/s) was reasonably constant. At shorter step-test intervals, the critical velocity increased rapidly to exceed 7 L/s at a step-test interval of 30 s. The cruising speed of control fish was estimated at 4.5 L/s.

The influence of fenitrothion exposure on swimming speed is related to previous studies on sublethal effects of fenitrothion and to concentrations in streams resulting from insecticide spraying.

PETERSON, R. H. 1974. Influence of fenitrothion on swimming velocities of brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 31: 1757–1762.

Les vitesses de nage critiques de l'omble de fontaine (*Salvelinus fontinalis*) après exposition à 150, 500 et 1500 μg/litre de fenitrothion, sont respectivement de 100, 83 et 70% de la performance des poissons témoins. Ceci représente une diminution de 35 et 60% respectivement du rendement énergétique pour les deux concentrations les plus élevées.

A des intervalles de plus de 15 min d'essais par étapes, la vitesse critique (ca 4.5–5 longueurs [L]/sec) est raisonnablement constante. A des intervalles plus courts, la vitesse critique augmente rapidement pour dépasser 7 L/sec à un intervalle de 30 sec. La vitesse de croisière des témoins est estimée à 4.5 L/sec.

Nous faisons la relation entre l'influence de l'exposition au fenitrothion sur la vitesse de nage et des études antérieures sur les effets sublétaux du fenitrothion, et les concentrations observées dans les cours d'eau à la suite d'arrosages de l'insecticide.

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FENITROTHION, an organophosphate insecticide, is used in forest spraying to control spruce budworm in New Brunswick, Canada. It is sprayed in spring and early summer when streams are usually discharging large volumes of water. Although spraying at that time results in maximal dilution of the insecticide, associated higher stream velocities could affect survival of exposed fishes through reduction of swimming performance. Swimming performance has been utilized or suggested for use as an important criterion in the determination of the sublethal effects of various pollutants on fish (Brett 1967a; Sprague 1971). Ability to maintain position in streams, to escape predators and to catch food all require swimming capability which may be altered by exposure to various chemicals. Cyanide (Neil 1957), detergents (Cairns and Scheier 1962), pulpwood fiber (MacLeod and Smith 1966), sodium pentachlorophenate (Webb and Brett 1973), and hydrogen sulfide (Oseid and Smith 1972) are some of the substances found to affect swimming performance.

Two series of experiments were performed to determine whether fenitrothion affected swimming performance of brook trout (*Salvelinus fontinalis*) under exercise regimes which utilized the various types of swimming from "spurt" to "sustained" (Reimers 1956; Bainbridge 1962; Brett 1967b). The effects of fenitrothion on both velocity and calculated power are considered.

Methods

A Blâzka-type respirometer (Blâzka et al. 1960) with an inner chamber 112 cm long and 12.5 cm in

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diameter was used to exercise the fish. An electrified wire grid screened the inner chamber just in front of the propeller, with the voltage kept at the minimum required to cause fish to swim upstream away from the grid. The ventral, anterior half of the apparatus was surrounded by a series of black and white stripes to provide visual reference. The chamber was covered with black plastic, except for the 30-cm section immediately in front of the grid. Only light from the slightly opened laboratory doorway was used to illuminate the room. Tests were conducted at the 15 C acclimation temperature. Aerated water at that temperature flowed through the respirometer continuously at ca 2 liters/min.

Water current flow in the respirometer was calibrated with a pilot-static tube and with an "Ott" propeller-type current meter. The results from the two methods agreed within 5%. Response time following an increase in motor speed was slightly less than 2 s. This resulted in a lag of 5–15% at step-test intervals of 10–30 s, but an insignificant error at longer intervals.

Brook trout (*S. fontinalis*) 8–12 mo of age (and with standard length of 8.3–12.6 cm) were used. Condition factor (CF) of the fish didn't vary significantly throughout the experiments (mean CF — 1.40, range 1.09–2.01). Standard length was used to calculate the CF.

Initially, large fish were selected for experiments, so that the effect of growth during the experimental period was minimized. Nevertheless there was some increase in size, fish having mean body lengths of ca 9 cm initially and about 11–12 cm by the end of the experiments. The mean weight of all fish tested was 15.8 g, with a range of 7.0–26.0 g.

The experiments were performed in two series: series 1 — from October 4, 1972, until April 19, 1973; and series 2 — from September 19, 1973, until January 16, 1974. Fish of the previous spring's hatch were used in each series.

Fish were exposed singly under static water conditions to technical grade fenitrothion solubilized in a nonionic detergent (Corexit® 7664) for 24 h in 10 liters of aerated water at 15 C. Control fish received an identical exposure to the detergent (0.1 ml/10 liters). In series 1 the concentration of fenitrothion was varied from 0.15 to 1.5 ppm and the exercise regimen was constant. In series 2 the concentration was 0.5 ppm and the exercise regimen was varied.

The fenitrothion, a Bayer product, was obtained from Forest Protection Limited, Fredericton, N.B. It contained several impurities of which one was S-methyl fenitrothion (Zitko and Cunningham 1974). The stock solutions were replaced every 2 mo and were kept refrigerated in darkness.

Following exposure the fish were placed in the respirometer for either 2 or 24 h before they were exercised. At the end of the rest period, the fish were exercised at an initial velocity of 18 cm/s, and the velocity was increased stepwise in increments of 3 cm/s until the fish fatigued. In series 1 the time interval between velocity increments (henceforth called the "step-test interval") was 0.5 h, while in series 2 the step-test interval was varied from 10 s to 12 h in different experiments. The highest velocity at which the fish were able to swim for the full step-test interval was accepted as the "critical" velocity under the conditions of the particular test.

Critical velocities (Brett 1967a) as determined in these experiments are slightly underestimated. The time the fish swam at the highest velocity before becoming fatigued and falling back on the grid was not considered.

In series 1, tests with control and treated fish were alternated, and tests at one fenitrothion concentration were completed before the next higher concentration was used. In series 2, the step-test intervals utilized were varied at random between experiments, and controls and treated fish were paired. Thus, any seasonal effects or growth effects were minimized.

The critical velocity achieved by each fish was adjusted, for purposes of standardization, to the equivalent maximum velocity of a fish of 10 cm body length (L) by means of the formula $V_1 = \sqrt{10V^2/L}$ (Smit et al. 1971). Since most of the fish were used within 2 cm of this value, the adjustment was small. The maximum power was then calculated using the formula $P = 0.75\ \rho\ A(V_1)^3C$, where ρ = viscosity of water, A is the surface area of the fish ($0.4\ L^2$), V_1 is the corrected velocity and C is the drag coefficient (Smit et al. 1971).

The probit analysis method suggested by Brett (1967a) for calculation of the 50% fatigue time was not utilized as variation in performance among fish was small. However, variability tended to increase with dosage among treated fish. Therefore the mean critical velocity was considered a suitable parameter for comparison of swimming capability.

Results

Series 1 — Critical velocities attained by brook trout after 24-h exposure to fenitrothion were lower than those of the controls (Fig. 1). The degree to which the critical velocity was lowered after fenitrothion exposure was a function of fenitrothion concentration and length of the rest period prior to exercise.

Fenitrothion at 1500 µg/liter is near the 24-h LC50 for these trout, and about 25% of the fish died during the 24-h exposure. The surviving fish were markedly sluggish when first placed in the respirometer. After a 24-h rest period in the respirometer, however, the treated fish showed only a small though statistically significant (Table 1) decrease in critical velocity when compared with the performance of control fish (Fig. 1, asterisk). Furthermore, when the treated fish were removed from the respirometer at the end of an experiment, they were much livelier than when first placed in it. Obviously, some recovery occurred during the experiment of 5–6-h duration and the preceding 24-h rest period. The rest period was therefore reduced to 2 h to avoid recovery. The shortened rest period apparently had little effect on the performance of

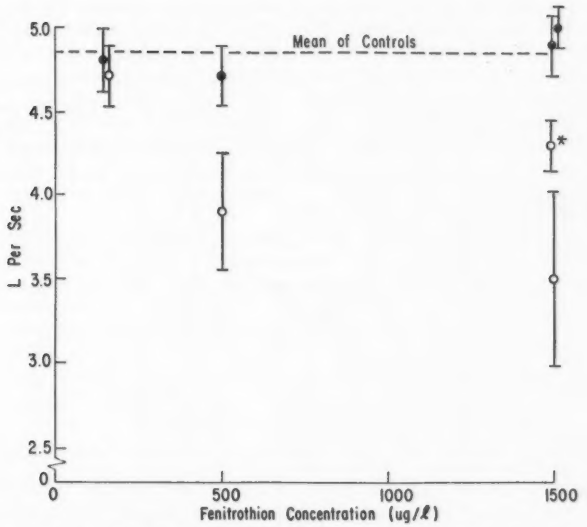

FIG. 1. Critical swimming velocities ±2 SE (series 1) for controls (●) and for fish exposed to various concentrations of fenitrothion (○). Standard rest period between fenitrothion exposure and beginning of test — 2 h (* — 24-h rest period). One ppm fenitrothion is equivalent to 1000 μg/liter.

control fish as the mean critical velocity after 2 h of rest was 5.0 L/s as compared to 4.9 L/s after 24 h of rest (Fig. 1). The treated fish with only 2 h rest did not swim as well; their mean critical velocity was 3.5 L/s, representing a 30% decrease in velocity and a 60% decrease in power. A 2-h rest period following dosage was used for all subsequent experiments to minimize recovery from fenitrothion exposure.

TABLE 1. The values of t and probabilities of null hypothesis for differences in mean critical velocities between controls and fish exposed to fenitrothion. In series 1 the step-test interval was constant at 30 min. In series 2 the fenitrothion concentration was constant at 0.5 ppm. Rest period was 2 h, except where otherwise indicated.

		df	t	P
Series 1	1.5 ppm, fenitrothion, 24-h rest	18	5.27	<0.001
	1.5 ppm, fenitrothion	10	5.24	<0.001
	0.5 ppm, fenitrothion	10	3.88	<0.005
	0.15 ppm, fenitrothion	10	1.37	ns
Series 2	60-min step-test interval	10	0.09	ns
	30 min " " "	10	2.67	<0.025
	15 min " " "	10	5.59	<0.001
	5 min " " "	10	2.57	<0.025
	1.5 min " "	10	1.56	ns
	0.5 min " "	10	3.26	<0.01

Exposure to 500 μg/liter fenitrothion resulted in 17 and 35% decreases in velocity and power, respectively. Exposure to 150 μg/liter fenitrothion resulted in no significant change in critical velocity (Fig. 1), so that the threshold for reduction in velocity is near 100 μg/liter.

Series 2 — The experimental variable in this series was the duration of the step-test interval (from 30 s to 1 h). Six controls and six fish exposed to 500 μg/liter fenitrothion were tested at each step-test interval (Table 2).

The duration of the step-test interval had only a slight effect on mean critical velocity for treated or control fish at intervals longer than 30 min (Fig. 2A). The curve becomes asymptotic at step-test intervals beyond approximately 60 min. The asymptote yields an estimate of the cruising speed (defined as the maximum velocity a fish can sustain for very long periods, Fry and Hart 1948). The cruising speed of these trout would be near 4.5 L/s.

Exposure to 500 μg/liter fenitrothion resulted in a small, but consistent decrease of ca 11% in critical velocity at step-test intervals of 30, 15, 5, 1.5, and 0.5 min (Fig. 2A). The differences between test and control means differed significantly except for the

TABLE 2. Means and ranges of body lengths and mean experimental durations for fish used under various experimental conditions. All exposures to fenitrothion were for 24 h and the rest period was 2 h unless noted otherwise. Step-test interval is the time between velocity increases.

Series no.	Fenitrothion concn	Step-test interval (*min*)	N	Body length		Mean experimental duration (*min*)	Critical velocity (L/s)
				Mean	Range		
1	1.5 ppm (24-h rest)	30	10	9.6	(9.0–12.0)	276	4.34
	Control "	30	10	9.9	(8.6–10.8)	342	4.94
	1.5 ppm (2-h rest)	30	6	9.9	(9.0–10.6)	205	3.50
	Control	30	6	10.4	(10.0–10.9)	355	4.95
	0.5 ppm	30	6	9.6	(9.0–10.1)	235	3.90
	Control	30	6	9.9	(9.6–10.5)	310	4.67
	0.15 ppm	30	6	10.2	(9.4–11.5)	320	4.67
	Control	30	6	10.5	(10.0–11.4)	345	4.83
2	0.5 ppm (2-h rest)	60	6	10.6	(8.6–11.7)	660	4.62
	Control	60	6	10.4	(9.1–11.3)	640	4.63
	0.5 ppm	30	6	10.6	(9.6–12.2)	315	4.50
	Control	30	6	10.0	(8.3–11.0)	345	4.93
	0.5 ppm	15	6	10.8	(9.4–11.7)	152.5	4.37
	Control	15	6	10.9	(10.2–11.9)	192.5	5.13
	0.5 ppm	5	6	10.1	(8.0–11.4)	57	4.87
	Control	5	6	10.6	(9.4–11.4)	68	5.42
	0.5 ppm	1.5	6	11.0	(9.9–12.0)	21.5	5.72
	Control	1.5	6	11.0	(10.6–11.6)	25.2	6.17
	0.5 ppm	0.5	6	11.0	(10.1–11.8)	9.1	6.62
	Control	0.5	6	11.6	(10.6–12.6)	11.2	7.65
	Control	0.167	1	11.2		4.3	8.30
	Control	720	1	11.1		7920	4.33

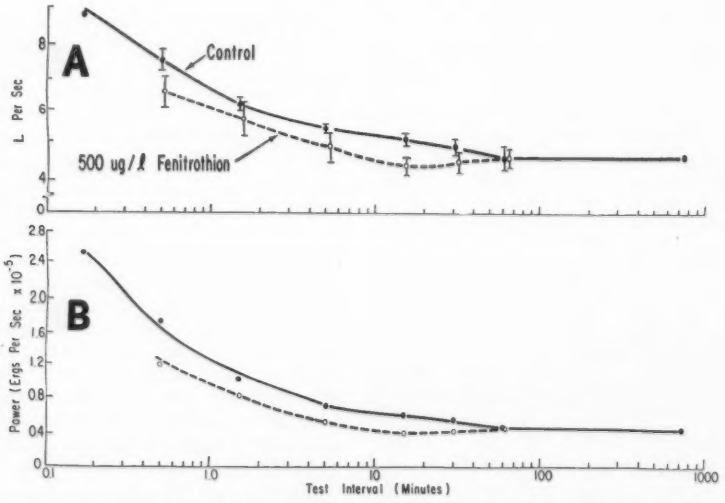

FIG. 2. A, Critical swimming velocities ±2 SE (series 2) at various step-test intervals for control fish (●) and treated fish (○). The curves are fitted by eye. B, Power generated at the critical velocities of Fig. 2A are shown for various step-test intervals.

step-test interval of 15 min (Table 1, series 2). The deficits in critical velocities induced by fenitrothion exposure were not as large as were obtained with 500 μg/liter fenitrothion in series 1 (17%). At a step-test interval of 60 min, the mean critical velocities for test and control groups were identical (Fig. 2A). The duration of these experiments with 60-min step-test intervals was about 12 h, so that recovery may have occurred before the critical velocity was attained.

The maximum power output rose sharply at the shorter step-test intervals (Fig. 2B). The maximum power generated by the single fish tested at the 0.16-min step-test interval was 2.5×10^5 ergs/s — about 6 times the power required at the cruising speed. The mean percent decrease in power, at step-test intervals shorter than 0.5 h due to fenitrothion exposure, was about 35%.

Discussion

Maximum concentrations of fenitrothion found in New Brunswick streams as a result of insecticide spraying are usually within the range of 5–20 μg/liter (Banks and Hall 1972; Hall and Eaton 1973; Peterson and Zitko 1974). The time interval over which significant levels of fenitrothion are present in streams varies from a few hours to several days after spraying. Later peaks in concentration may occur as a result of runoff after rain. Generally speaking, maximal concentrations of fenitrothion in sprayed streams are usually an order of magnitude below the threshold where swimming performance of brook trout (of the size tested) is affected. Côté and Tétreault (1973) reported large downstream movements of salmon parr after fenitrothion spraying of Anticosti Island. It seems possible, however, that these movements were associated with sudden large increases in stream velocity from rains rather than from spray effects.

Exposure to 1000 μg/liter fenitrothion eradicated learning by Atlantic salmon (*Salmo salar*) (Hatfield and Johansen 1972) and increased vulnerability of Atlantic salmon parr to predation by brook trout (Hatfield and Anderson 1972). However, in neither instance was there an effect at 100 μg/liter. Recovery of learning ability at 1000 μg/liter was partial in 4 days and complete in 7 days postexposure. Exposure of Atlantic salmon parr to 1000 μg/liter fenitrothion also had been found to decrease the ability to hold territory in an artificial stream (Symons 1973). Exposure to 100 μg/liter caused a lesser reduction in territory-holding ability, and recovery occurred in 2–3 wk. It was also noted that fish subjected to the higher dose swam "stiffly" at first, but normal swimming occurred after 24 h. Wildish and Lister (1973) found that fenitrothion affected dominance hierarchy and food conversion efficiency in brook trout at 10,000 μg/liter but not at 1000 μg/liter. The fenitrothion was fed to the trout in their food, so that simple comparisons between their method of dosing and the method used here cannot be made. Recovery again occurred in 2–3 wk, as did recovery of brain acetylcholinesterase.

The evidence suggests that the threshold of sublethal effects for salmonids is near 100 μg/liter of fenitrothion in the water. Recovery rates vary, and there is a suggestion that complex behavior such as learning and territoriality require longer recovery periods (1–3 wk) than does recovery of swimming ability (24 h or less).

Decrease in critical velocity following exposure to fenitrothion could be due to direct action on muscle, effects on areas of the nervous system concerned with muscle activity, or indirect effects through motivational disturbance. Fenitrothion is a known inhibitor of brain acetylcholinesterase (Wildish and Lister 1973; O'Brien 1967). However, it is difficult to relate decrease in swimming performance to acetylcholinesterase inhibition; the rate of recovery of brain acetylcholinesterase is much slower than recovery of swimming ability. However, blood plasma cholinesterase levels, inhibited by organophosphates other than fenitrothion, do recover within 24 h (Pickering and Pickering 1971). Convulsions, which are a sympton of organophosphate poisoning in mammals, were absent in trout used in the experiments described here. Decrease in blood pressure is associated with organophosphate poisoning in mammals (O'Brien 1967), and similar decreases in fish could affect swimming performance.

The critical velocities attained at the two longest step-test intervals (60 and 720 min) differed little (assuming the one fish tested at the 12-h step-test interval is representative); it is assumed they are near the cruising speed of these fish, namely about 4.5 L/s. This compares reasonably well with cruising speeds given for salmonids elsewhere in the literature (Bainbridge 1960; Griffiths and Alderdice 1972; Brett 1967a). The fish used in experiments described here were not exercised prior to experimentation, and the cruising speed probably could be improved with training (Greer Walker and Pull 1971). At step-test intervals shorter than 15 min, the critical velocity increases steadily. It is probable that this is due to increasing use of the white muscle so that the very shortest step-test intervals yield data on "spurt" velocities. According to the equations of Bainbridge (1958) relating velocity to tail-beat frequency, a rainbow trout (*S. gairdneri*) of 10 cm should attain a maximum spurt velocity of about 15 L/s. Values approaching Bainbridge's figure are

obtained if the control curve in Fig. 2A is extrapolated to very short step-test intervals (assuming that the semilogarithmic relationship is linear at step-test intervals shorter than 1.5 min). For example, at step-test intervals of 0.01 min (0.6 s) the estimated critical velocity is 12.5 L/s.

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Apparent Specific Dynamic Action of Largemouth Bass, *Micropterus salmoides*

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Beamish, F. W. H. 1974. Apparent specific dynamic action of largemouth bass, *Micropterus salmoides*. J. Fish. Res. Board Can. 31: 1763–1769.

Oxygen consumption attributable to apparent specific dynamic action (SDA) was measured in relation to feeding level and body weight in largemouth bass, *Micropterus salmoides*, forced to swim at 1.7 body lengths/s (BL/s). Apparent SDA rose curvilinearly with ration size, the coefficient increasing with weight of bass. For a ration of fixed percent body weight/day, apparent SDA increased curvilinearly with weight of bass, the rate being most pronounced at higher levels of food intake. Apparent SDA expressed in energy units as a percent of ration ingested did not differ significantly with weight of fish or meal size. The overall mean apparent SDA ± SD was 14.19 ± 4.19% of the energy ingested. Time required for oxygen consumption to subside to pre-feeding levels increased with ration size and weight of bass.

Over the range of swimming speeds from 1.4 to 2.5 BL/s, there were no differences in apparent SDA of similar size bass fed a ration of 4% body weight/day. Similarly, time for elevated levels of oxygen consumption to return to prefeeding rates was independent of swimming speed.

Beamish, F. W. H. 1974. Apparent specific dynamic action of largemouth bass, *Micropterus salmoides*. J. Fish. Res. Board Can. 31: 1763–1769.

Nous avons mesuré la consommation d'oxygène attribuable à la SDA (action dynamique spécifique) apparente, en fonction du niveau d'alimentation et du poids corporel, chez des achigans à grande bouche, *Micropterus salmoides*, forcés de nager à une vitesse de 1.7 longueur du corps/sec (BL/sec). La SDA apparente augmente en fonction curvilinéaire du volume de la ration, le coefficient augmentant avec le poids de l'achigan. Pour une ration de pour cent fixe du poids corporel/jour, la SDA augmente en fonction curvilinéaire du poids de l'achigan, le taux étant plus prononcé aux niveaux de ration alimentaire plus élevés. La SDA apparente, exprimée en unités d'énergie sous forme de pourcentage de ration ingérée, ne change pas significativement avec le poids du poisson ou le volume de la ration. La moyenne générale de la SDA apparente ± l'écart type est de 14.19 ± 4.19% de l'énergie absorbée. Le temps nécessaire à la consommation d'oxygène pour s'abaisser aux niveaux d'avant l'alimentation augmente avec le volume de la ration et le poids de l'achigan.

Sur la gamme de vitesse de nage de 1.4–2.5 BL/sec, il n'y a pas de différences de SDA apparente chez des achigans de même taille et nourris d'une ration de 4% du poids corporel/jour. Pareillement, le temps nécessaire aux niveaux élevés de consommation d'oxygène pour retourner aux taux d'avant l'alimentation est indépendant de la vitesse de nage.

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It was early demonstrated for higher vertebrates that following ingestion of a meal, metabolism, expressed in units of heat production, increased. This increase was termed specific dynamic effect by Rubner (1902), later inappropriately translated as specific dynamic action (SDA), and is also known as calorigenic effect. The chemistry of SDA is not completely understood but the energy liberated is generally assumed to be largely the result of the deamination of amino acids. The liver appears to be the primary if not the only site of heat production (Buttery and Annison 1973). Although heat production is most pronounced with protein (Krebs 1964), some is released following ingestion of carbohydrate and fat. If each component is fed separately their respective heat production in homeothermic animals would be equivalent to 30% of the caloric content of protein, 13% for lipid, and 5% for carbohydrate (Harper 1971). For a

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composite diet including protein, lipid, and carbohydrate the total production of heat is less than that calculated from the nutritional composition of the feed (Forbes and Swift 1944).

Energy requirements for absorption, digestion, transportation, and deposition of food materials, while presumed to be minimal, are distinct from those for SDA but are difficult to separate experimentally. Where the distinction is not made, the term apparent SDA or heat increment is appropriate. Particularly little is known of apparent SDA in fish, notable exceptions being the measurements made by Averett (1969) on coho salmon, *Oncorhynchus kisutch*, and by Muir and Niimi (1972) on aholehole, *Kuhlia sandvicensis*.

In the present study apparent SDA was measured for different size largemouth bass, *Micropterus salmoides*, forced to swim in a tunnel respirometer (Farmer and Beamish 1969). The influence of various feeding levels of lake emerald shiners, *Notropis atherinoides*, and swimming speeds on apparent SDA were determined.

Materials and Methods

Largemouth bass were captured by seine from a small lake near Shakespeare, Ontario and transported to the laboratory where they were held in aerated, nonchlorinated, well water (total hardness 360 mg/liter as $CaCO_3$; pH 8.3). Fish were maintained for several months at 20 C (temperature at time of capture) and fed to satiation once daily on freshly thawed lake emerald shiners with a weekly supplement of beef liver. Photoperiod was maintained at 16 h of light throughout the study with a gradual change in intensity for 1/2 h at each end of the light regime. Maximum light intensity at the surface of the uncovered portion of the holding tanks was 60 lx. Before fish were used in an experiment they were acclimated to 25 C by a daily change of one degree and held 1 mo at the desired temperature. Ammonia nitrogen generally was below 0.1 mg/liter and oxygen above 8 mg/liter.

Apparent specific dynamic action (SDA) was determined for bass forced to swim in a respirometer (Farmer and Beamish 1969). Briefly, water in the respirometer is driven by a centrifugal pump into an expansion chamber in which are located a series of flow "straighteners," through a reduction cone, and into the portion of the chamber where bass are forced to swim. Velocity profile in the swimming chamber was rectilinear and essentially constant. For the present study the respirometer was modified to permit a continuous and constant flow of water through the chamber. Rate of flow was adjusted for each bass to provide sufficient oxygen (Beamish 1970) and to permit detection of differences in oxygen concentration between the inflow and outflow. Oxygen content of the outflow water was recorded continuously (YSI Oxygen Meter, Model 54) with regular checks being made on the inflow which generally remained constant and near air saturation. Bass were weighed ($\pm$ 0.1 g) before they were placed in a respirometer.

Following introduction of bass into a respirometer oxygen consumption fluctuated considerably, presumably due to the stress of handling (Black 1958), but after 14–18 h of swimming little change was noted. In one experiment apparent SDA was determined for six bass of 65.5 g mean weight (range 59.0–74.4 g) forced to swim at each of three speeds, 1.4, 1.9, and 2.5 BL/s. At each velocity bass were fed a ration of 4% body weight/day. In a second experiment, velocity was adjusted according to the size of bass (Beamish 1970) to maintain 1.7 BL/s (range 1.5–1.9) and feeding level varied from 2 to 8% body weight/day in increments of 2%. Number of bass examined at each feeding level varied from 15 at 4% body weight/day to 8 at 8%, the overall total being 46. The weight range of bass used was about the same for each feeding level with an overall mean ($\pm$ SD) of 64.0 $\pm$ 48.5 g. The maintenance ration of emerald shiners has been estimated by Niimi and Beamish (1974) to be 1.5% body weight/day for largemouth bass. The usual practice was to begin with 2% and proceed towards 8%; however, replications of a given feeding level were sometimes made. Bass were always fed between 0900 and 1000.

Fish were forced to swim for at least 24 h before being offered food. The feeding procedure was first to stop the current in the respirometer and then, with forceps, to offer the prescribed ration of emerald shiners to the bass through a vertical tube mounted on the swimming portion of the chamber. Generally, fish took the food immediately. If the entire ration was not eaten at once the uneaten portion was removed and the fish exercised for an additional day when the procedure was repeated. If a fish did not eat the prescribed ration on the second trial it was removed from the respirometer; however, this occurred only for two of the more than 60 bass used. Once a fish fed, the velocity was resumed and oxygen consumption monitored until it returned to the pre-feeding level, whereupon the fish was again fed.

The increase in oxygen consumption following ingestion of a prescribed ration was summed and equated to apparent SDA and the feeding procedure itself. An estimate of the metabolic expenditure associated with the feeding procedure was made for 12 bass (8.8–190.0 g) after they were trained to accept food from forceps. The entire feeding procedure was followed except that food was withheld. Immediately after a fish bit at the empty forceps the velocity was resumed and oxygen consumption monitored until it returned to the pre-feeding level.

The experiment on the influence of bass weight and ration size on apparent SDA was designed as a mixed model (Steele and Torrie 1960). Multiple regression analysis, with an error mean square including both pure error and lack of fit, was applied to determine if the rate of change of the dependent variable with ration size and bass weight was constant. The same analysis was applied to the influence of bass weight and ration size on the time for elevated levels of oxygen consump-

tion following ingestion of a meal to subside to pre-feeding levels.

Emerald shiners were collected from Lake Erie during early December, packaged in polyethylene bags within several hours of capture, and frozen (–18 C). Proximate analyses of freshly thawed shiners were made throughout the year. Moisture was removed from samples by lyophilization. Samples were then homogenized and protein N determined by the micro-Kjeldahl methods of Fels and Veatch (1959). Lipid analyses were performed by the chloroform–methanol wet extraction technique of Folch et al. (1957). Caloric content of dried samples was measured with a microbomb calorimeter (Phillipson 1964). In accord with the recommendation of the Association of Official Analytical Chemists (1970) samples were ashed at 550 C to constant weight.

Results

Oxygen consumption of largemouth bass forced to swim at a fixed speed increased abruptly after feeding to a maximum and thereafter decreased gradually, or in some cases irregularly, to pre-feeding levels. Time required to reach maximum oxygen consumption following a meal increased with meal size from a mean ($\pm$ SD) of 6.0 $\pm$ 3.3 h at 2% body weight/day to 13.3 $\pm$ 3.3 h at 8%. Within the weight range of bass used at each ration, differences in time to reach maximum oxygen consumption were not demonstrable.

Ambient oxygen in the respirometer was not allowed to fall below 70% of air saturation. Oxygen consumption of bass at this level does not differ appreciably from that above air saturation, irrespective of swimming speed (Beamish 1970). Maximum oxygen consumption following ingestion of a meal generally fell short of the metabolic rate associated with maximum sustained swimming (active) for bass in the postabsorptive stage. The mean maximum metabolic rate associated with feeding levels of 2% body weight/day represented 80.9% of active oxygen consumption. This increased to 96.5% following feeding levels equivalent to 6% body weight/day. At the highest ration fed, 8% body weight/day, maximum oxygen consumption coincided with the active metabolic rate. Generally, maximum rates of oxygen consumption were maintained only for 1–2 h.

Time required for oxygen consumption to subside to pre-feeding levels increased with ration size and weight of bass. This relationship, examined by multiple regression analysis, suggested the equation best describing the response surface also included interaction terms between bass weight and ration size. Only those variables with t-values significant at $P < 0.05$ were accepted. The relationship is described by the equation:

$$T = 1.99 \times 10 + 1.37 \times 10R - 6.50 \times 10^{-1} R^2 - 2.50 \times 10^{-1} W + 1.32 \times 10^{-3} W^2 - 6.61 \times 10^{-2} RW + 4.06 \times 10^{-3} R^2W$$

where

	SE	t-value
T is time in h		
R is ration in g	2.2901	6.0000*
R^2	0.1046	6.2100*
W is weight of bass in g	0.0819	3.0500*
W^2	0.0006	2.1200*
RW	0.0259	2.5500*
R^2W	0.0013	3.1200*

*$P \leq 0.05$

The error mean square and error degrees of freedom are 31.508 and 43, respectively, and the multiple R-squared, 0.799.

The curvilinear relationship between time required for oxygen consumption to subside to pre-feeding levels and ration size was also dependent on bass weight (Fig. 1). Thus following the ingestion of a progression of rations between 2 and 8%, the period of elevated oxygen consumption increased from 20.4 to 34.2 h for bass of 25 g and from 27.4 to 72.6 h for fish of 200 g.

Oxygen consumption attributable to the feeding procedure itself increased to a maximum within about 2.0 h (range 1.0–3.5 h) and thereafter declined generally reaching the pre-feeding level

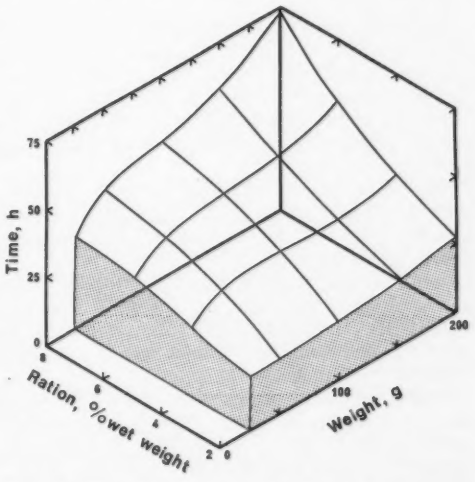

FIG. 1. Time for elevated rates of oxygen consumption to subside to pre-feeding levels in relation to ration size and weight of largemouth bass (*Micropterus salmoides*).

within 6 h. The total increase in oxygen uptake attributable to the feeding procedure increased curvilinearly with weight, the rate decreasing inversely with size of bass. This relation is described by the equation:

$$\log O_2 = 7.96 \times 10^{-1} + 8.56 \times 10^{-1} \log W$$

where O_2 represents oxygen consumption in milligrams and W, weight of bass in grams. The SE of the regression coefficient is 0.129 and SE of estimate, 0.131. The correlation coefficient, 0.931, with 11 df is significant at $P \leq 0.05$.

The total elevation in oxygen consumption for each fish following ingestion of a meal was corrected for that attributable to the feeding procedure from the above regression and equated to apparent SDA.

Apparent SDA, expressed as milligrams of oxygen consumed, varied with weight of bass and ration size and is described by the equation:

$$O_2 = 89.13 \times 10^{0}\ R - 12.45 \times 10^{-2}\ RW - 3820.57 \times 10^{-2}$$

where

	SE	*t*-value
O_2 is oxygen consumption in mg		
R is ration in g	7.1499	12.42*
W is weight of bass in g		
RW	0.0379	3.29*

*$P \leq 0.05$

The error mean square and error degrees of freedom are 2220.18 and 43, respectively, and the multiple R-squared, 0.948. Of the variation in oxygen consumption, 93.52% is explainable by ration alone and only 1.30% is attributable to the weight ration interaction which nevertheless was significant at $P \leq 0.05$.

The curvilinear relationship between apparent SDA and bass weight was also dependent on ration size (Fig. 2). Thus, over rations of 2–8%, apparent SDA of bass of 25 g increased from 4.8 to 133.8 mg and for fish of 200 g from 218.7 to 989.5 mg. For a ration of fixed size, percent wet weight, apparent SDA increased curvilinearly with size of bass, the rate being most pronounced at the lower levels of food intake.

Reproducibility of estimates of apparent SDA was tested on several occasions. Bass fed consecutive meals of similar size exhibited little fluctuation in apparent SDA. For example, apparent SDA's for a 70-g bass fed successive rations of 4%/day were 231.6, 229.8, and 234.0 mg.

Swimming speed at each of 1.4, 1.9, and 2.5 BL/s did not influence the pattern of elevated oxygen consumption following the ingestion of a meal of 4% body weight/day. Maximum oxygen consumption following feeding increased from 79.2% of the active metabolic rate at 1.4 BL/s to 99.7% at 2.5 BL/s. Time for elevated levels of oxygen consumption to return to pre-feeding rates was independent of swimming speed. Moreover, over the swimming speeds applied there was no suggestion of differences in apparent SDA (Fig. 3).

Proximate analyses of 88 individual freshly thawed emerald shiners were made throughout the year. For several samples of shiners, the geometric mean content of protein and lipid $\pm$ 2 SE was not within the respective overall mean $\pm$ 2 SE. Nevertheless it was presumed that variation among samples was in large part a function of sample size, and that the overall geometric means given in Table 1 best represented the proximate composition.

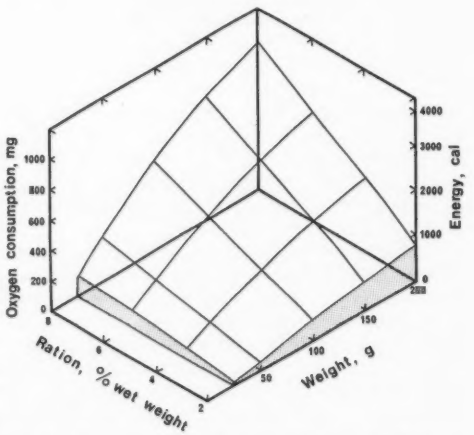

FIG. 2. Apparent specific dynamic action of largemouth bass, as oxygen consumption and energy, in relation to ration size and weight of fish.

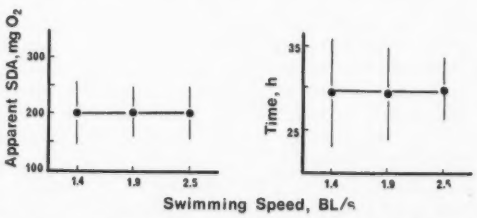

FIG. 3. Apparent SDA and time for elevated levels of oxygen to subside following ingestion of a meal of 4% body weight/day in relation to swimming speed. The mean of six largemouth bass is given along with the standard deviation.

TABLE 1. The geometric mean content of protein and lipid ± 2 SE in several samples of emerald shiners (*Notropis atherinoides*).

Wt of individual emerald shiner (± 2 SE)			Protein (± 2 SE)		Lipid (± 2 SE)	
Wet (*mg*)	Dry (*mg*)	%	mg	% organic dry wt[a]	mg	% organic dry wt[a]
1176±1450	293±421.4	24.1±1.3	156.3±8.1	61.8±9.4	129.5±34.6	35.6±1.6

[a]Brett and Higgs (1970).

Caloric and ash content, 6.460 ± 0.114 cal/mg organic dry weight and 10.8 ± 0.95% dry weight, respectively, were determined once for 20 individual shiners.

Discussion

Conversion of apparent SDA from units of oxygen consumption to the standard heat equivalent is based either on complete oxidation of the food or on the physiological equivalents of the metabolized constituents. Energy equivalents can be applied only if the system is totally aerobic. In the present study oxygen consumption of bass forced to swim at 1.7 BL/s approached the active metabolic rate only after a meal of 8% body weight/day and then only for a period of about 1 h. When feeding level was maintained at 4% body weight/day but swimming varied between 1.4–2.5 BL/s maximum oxygen consumption increased to the active level only at the highest velocity applied. Obviously determinations of apparent SDA on exercised fish must be conducted with caution. Total metabolism is certain to contain an anaerobic fraction in the circumstance where fish are fed large meals and forced to swim at high speeds. Warren (1971) suggests SDA differs with level of activity but as demonstrated for bass it remains constant over a range of swimming speeds provided the feeding level is not high. Forcing fish to swim at a low and fixed speed offers the advantage that the metabolic expenditure for locomotion remains constant. Where fish are not forced to swim but rather are spontaneously active the energy attributable to locomotion must be continuously monitored.

Indications of anaerobic metabolism such as lactic acid production (Black 1958; Beamish 1968) or excessive respiratory quotients (Kutty 1968; 1972) were not measured in the present study. However, since active rates of oxygen consumption were approached only briefly after ingestion of the maximum rations fed particularly at the highest swimming speed applied it was presumed that anaerobic metabolism did not contribute appreciably to the general level.

The assignment of a precise oxycalorific equivalent is dependent on the proportions of carbohydrate, lipid, and protein catabolized. Winberg (1956) suggests that irrespective of the components oxidized the oxycalorific coefficient will not vary more than 1.5%. It is generally agreed an oxycalorific coefficient of 3.42 cal/mg oxygen consumed is most applicable for the higher vertebrates (Brody 1964). Recently Brett (1973) suggested this value is also acceptable for sockeye salmon, *Oncorhynchus nerka*.

Apparent SDA of bass, expressed in energy units as a percent of ration intake, did not differ significantly ($P > 0.05$) with weight of fish or meal size. The overall mean apparent SDA ± SD was 14.19 ± 4.19% of the energy ingested. Among the higher vertebrates SDA is sometimes expressed as a function of metabolizable energy, the difference between food energy and the cumulative fecal and urinary losses (Kleiber 1961). In fish, however, although nitrogenous products are excreted as urine significant amounts of diffusible products such as urea and ammonia are excreted across the gills. Hence the term urinary loss as applied to the higher vertebrates should be replaced for fish by nonfecal loss as suggested by Niimi and Beamish (1974). Fecal energy loss by bass has been estimated at 10.4% of the ingested energy when fed a diet of emerald shiners, irrespective of weight of bass or meal size (Beamish 1972). Nonfecal loss was calculated as 7.9% of ingested energy by Niimi and Beamish (1974). Thus metabolizable energy in bass represents 81.7% of that consumed. Apparent SDA accounts for 17.2% of the metabolizable energy.

Apparent SDA has not been estimated for many species of fish and in no case has the cost of feeding been deducted from the elevated level of oxygen consumption following ingestion of a meal. Undoubtedly the most thorough investigation on apparent SDA was that made by Averett (1969) on young coho salmon in relation to ration size,

season, and temperature. A diet of live housefly (*Musca domestica*) larvae was fed in amounts ranging from <1 to 20% of the caloric content of the salmon. Measurements of SDA expressed as percent of energy ingested, were sufficiently variable among individuals that an obvious relationship with season, temperature, or meal size was not demonstrable. Estimates ranged between 3.4 and 45.0% of the energy ingested with most values between 9.0 and 15.0%. Muir and Niimi (1972) determined SDA for two specimens of aholehole for which they obtained a value of 16% of the ingested energy of rations of tuna equivalent to 2.3 and 4.5% of the wet body weight. More recently Pierce and Wissing (1974) estimated the cumulative expenditure of energy attributable to SDA and spontaneous activity for bluegill sunfish (*Lepomis macrochirus*) fed to satiation on mayfly (*Hexagenia limbata*) nymphs. Estimates for the 12 fish examined ranged from 4.8 to 24.4% of the caloric content of the daily ration. Thus the value found in the present study for bass falls within the rather wide range found for other species.

The importance of sharply defining the dietary and environmental conditions under which SDA is determined was stressed by Brody (1945). Bass were fed in the present study on a natural diet of emerald shiners, which did not vary appreciably in proximate body composition throughout the year, together with a weekly supplement of beef liver for some months before an experiment was begun. On the basis of nutritional studies conducted on rainbow trout (*Salmo gairdneri*) by Lett (personal communication) this interval is sufficient for the proximate body composition to equilibrate with respect to food quality. However, prior to an experiment bass were fed to satiation rather than to a specific feeding level and this might have influenced SDA.

Duration of elevated levels of oxygen consumption in largemouth bass at 25 C was dependent on weight of fish and meal size. Averett (1969) observed that oxygen consumption of coho salmon of 1.2–6.4 g generally returned to pre-feeding levels within 24 h between 3 and 23 C, irrespective of meal size. Accordingly, for bass of comparable weight apparent SDA was complete by 24 h irrespective of meal size in the present study. Muir and Niimi (1972) found oxygen consumption of a 44 g aholehole at 23 C returned to pre-feeding levels only after 42 and 60 h when fed rations of 2.3 and 4.5% of wet body weight, respectively. This is considerably longer than that required by bass of similar size fed an equivalent weight of food.

Elevated levels of oxygen consumption by bass following consumption of food at the higher ration levels continued for a period similar to that recorded for the absorption of protein (Beamish 1972). The geometric mean absorption efficiency of protein was 95.7% irrespective of ration size between 2 and 8% body weight/day. For a 91-g bass the expenditure of energy attributable to apparent SDA continued for about 48 h after ingestion of a meal of 8%, not appreciably different than the 46 h required for the absorption of protein. At the lower ration sizes, however, absorption of protein required more time than was necessary for oxygen consumption to return to pre-feeding levels. Thus after ingestion of a meal of 2% body weight/day by a bass of 91 g, protein was absorbed in slightly less than 46 h whereas elevated levels of oxygen consumption associated with apparent SDA continued only for about 21 h. Absorption of protein was approximately 85% complete within the time required for the elevation in oxygen consumption after a meal of 2% body weight/day to return to pre-feeding levels. Time required for 85% absorption of protein after a ration of 8% was 30 h. However, the actual amount of protein remaining in the digestive tract after 85% absorption was 0.16 g for bass fed 2% and 0.65 g for those fed 8% body weight. Increases in oxygen consumption associated with the smaller amount of remaining protein would of course be more difficult to detect than those associated with the larger meal size. Further, a small portion of the protein was undoubtedly derived from material sloughed from the lining of the digestive tract. The equation applied to the relationship among ration size, time of elevated oxygen consumption, and weight of bass accounted only for 79.9% of the variation. If better account could be made of the variation perhaps the estimates of time for SDA would correspond more closely to those for absorption of protein.

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A Test of a Simple Nutrient Budget Model Predicting the Phosphorus Concentration in Lake Water[1]

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DILLON, P. J., AND F. H. RIGLER. 1974. A test of a simple nutrient budget model predicting the phosphorus concentration in lake water. J. Fish. Res. Board Can. 31: 1771–1778.

The total phosphorus budgets for a number of lakes in the Haliburton–Kawartha region of southern Ontario were measured over a 20-mo period. These data, combined with the lakes' morphometry and water budgets, were used to test a simple nutrient budget model similar to that proposed by Vollenweider (1969) purporting to predict the total phosphorus concentration in lakes. Except in the case of two very shallow lakes ($\bar{z}$ <1 m), the concentrations predicted by the model were very close to those measured in the lakes at spring overturn. Additional data from the literature supported the belief that this model could be used effectively for oligotrophic and mesotrophic lakes. Its value lies in the fact that quantitative changes in phosphorus loading can be interpreted in terms of changes in phosphorus concentration, which in turn, can be related to changes in parameters that reflect the lake's trophic state such as summer chlorophyll *a* concentration.

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Les auteurs ont mesuré, sur une période de 20 mois, les budgets de phosphore total d'un certain nombre de lacs de la région Haliburton–Kawartha de l'Ontario méridional. Les données obtenues, combinées avec la morphométrie et les budgets hydrologiques des lacs, ont été utilisées pour évaluer un modèle simple de budget nutritif, semblable à celui que propose Vollenweider (1969) et visant à prédire la concentration du phosphore total dans les lacs. Sauf pour deux lacs très peu profonds ($\bar{z}$ < 1 m), les concentrations prédites par le modèle se rapprochent de très près de celles mesurées dans les lacs au moment du brassage général de printemps. Des données supplémentaires tirées de la littérature corroborent la croyance que ce modèle peut être utilisé efficacement dans le cas de lacs oligotrophes et mésotrophes. Sa valeur réside dans le fait que les changements quantitatifs de la charge de phosphore peuvent être interprétés en termes de changements de concentration du phosphore qui, à leur tour, peuvent être reliés aux changements dans les paramètres qui reflètent l'état trophique du lac, telle la concentration estivale de la chlorophylle *a*.

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To make wise decisions about lake management, it is not enough to know merely that more phosphorus entering a lake will increase the growth of algae. One also needs to know the quantitative relation between nutrient loading and algal abundance. That a relation probably exists was shown by Sakamoto (1966) who found that, provided the N to P ratio is greater than 12, the mean chlorophyll content of lake water is closely correlated with concentration of total phosphorus. If it were also possible to relate phosphorus concentration to existing or predicted phosphorus loading, then the particular prediction necessary for lake management would be possible.

A model relating phosphorus concentration at spring overturn to phosphorus loading, sedimentation rate, and water replenishment rate was proposed by Vollenweider (1969), and it appeared to work well when applied to the few oligotrophic lakes for which adequate data were available. Results for several eutrophic lakes were, however, less

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satisfactory. Although the need to measure sedimentation rate makes Vollenweider's model cumbersome to use, its conceptual simplicity and obvious potential advantages make it an excellent starting point from which to develop a model in which all terms are easily measured.

To formulate his model, Vollenweider made four assumptions about nutrients in lakes. First, he assumed that sedimentation rate of a substance is proportional to the amount of that substance in the lake. Second, he assumed that any substance is completely mixed throughout the entire lake as soon as it enters. This is obviously violated, particularly during summer stratification, when mixing between the epilimnion and hypolimnion is prevented by a thermal gradient. He also assumed that the concentration of a substance in the outflow is equal to the concentration in the lake and finally that there are no seasonal fluctuations in loading rate. In summary, his model treats a lake as a single-compartment, open system. From these assumptions it follows that at steady state,

$$[P] = \frac{L}{\bar{z}\,(\sigma + \rho)} \tag{1}$$

where [P] is the concentration in g m^{-3} of total phosphorus in the water, L is the phosphorus loading in g m^{-2} yr^{-1}, $\bar{z}$ is the lake's mean depth in meters, σ is the sedimentation rate coefficient as a fraction of the total P content sedimented yearly, and ρ is the replenishment coefficient or the fraction of the water volume lost annually through the outflow. To test this model, Vollenweider compared the predicted total phosphorus concentration with that measured at spring overturn, when, theoretically, the lake's composition is uniform. All of the terms except σ can be easily measured, and if some parameter related to σ, such as the fraction of the total P entering the lake which is retained in the lake, i.e. the retention coefficient, could be substituted for σ in the model, it could be much more useful. This is easily done with the steady state solution of Vollenweider's model. Under the same assumptions as were used to derive the model, it can be shown that the retention coefficient, R, a much more easily measured parameter, can be expressed as:

$$R = 1 - \frac{\rho[P]}{L/\bar{z}} = 1 - \frac{\rho}{\rho + \sigma} \tag{2}$$

Therefore

$$\sigma = \frac{R\,\rho}{1-R} \tag{3}$$

The model, of course, cannot be used to make this calculation, otherwise a tautology results. An independent means of calculating R is necessary:

$$R_{exp} = 1 - \frac{q_o [P]_o}{\Sigma q_i [P]_i} \tag{4}$$

where q_o is the outflow discharge volume ($m^3 yr^{-1}$), $[P]_o$, the outflow concentration, q_i, the inflow volumes, and $[P]_i$, the inflow concentrations.

Thus equation (1) can be rewritten as:

$$[P] = \frac{L\,(1-R_{exp})}{\bar{z}\,\rho} \tag{5}$$

In this variation of Vollenweider's model all of the terms are measured. Our objective was to measure P loading, the P retention coefficient, mean depth, and the replenishment coefficient in a series of lakes in southern Ontario to test the ability of equation (5) to predict the concentration of total P at spring overturn.

Materials and Methods

The lakes used in our study were all part of, or close to the Gull River drainage system in southern Ontario (Fig. 1). They were selected to provide the greatest variation in the parameters in Vollenweider's model (phosphorus loading, flushing time, mean depth) within a reasonably small area. A series of 18 lakes with surface areas from 1 to 13 km^2, watershed areas from 7 to 3500 km^2, and mean depths from 0.7 to 27 m was chosen. Nine lakes were in Precambrian granite or gneiss, the bedrock typical of the Canadian Shield, five were in Precambrian sedimentary rock, and four in Ordovician dolomitic limestone. In two instances a group of three lakes, Twelve Mile–Little Boshkung-Boshkung and Haliburton–Oblong-South Bay, and in one instance a pair of lakes, Eagle and Moose, were joined by broad, deep channels. Since we found it impractical to measure net water or P movement through these channels we treated each group of lakes as a single lake.

Watershed areas and lake surface areas were read from 1:500,000 topographic maps. All lakes were echo-sounded with a Furuno model FG-200 instrument to provide contour maps from which detailed morphometric data were obtained. These maps are on file in the map library of the University of Toronto, and morphometric data are presented by Dillon (1974).

Discharge of affluents and effluents was measured over 20 mo by a variety of techniques including current meter measurements at 20 and 80% of the water depth across a surveyed cross section of stream, dye dilution, rectangular and 90° V-notch weirs, and, for larger rivers, by the formulae for broad-crested weirs to obtain discharge at dam sites. Although continuous recorders were not used, most streams were visited

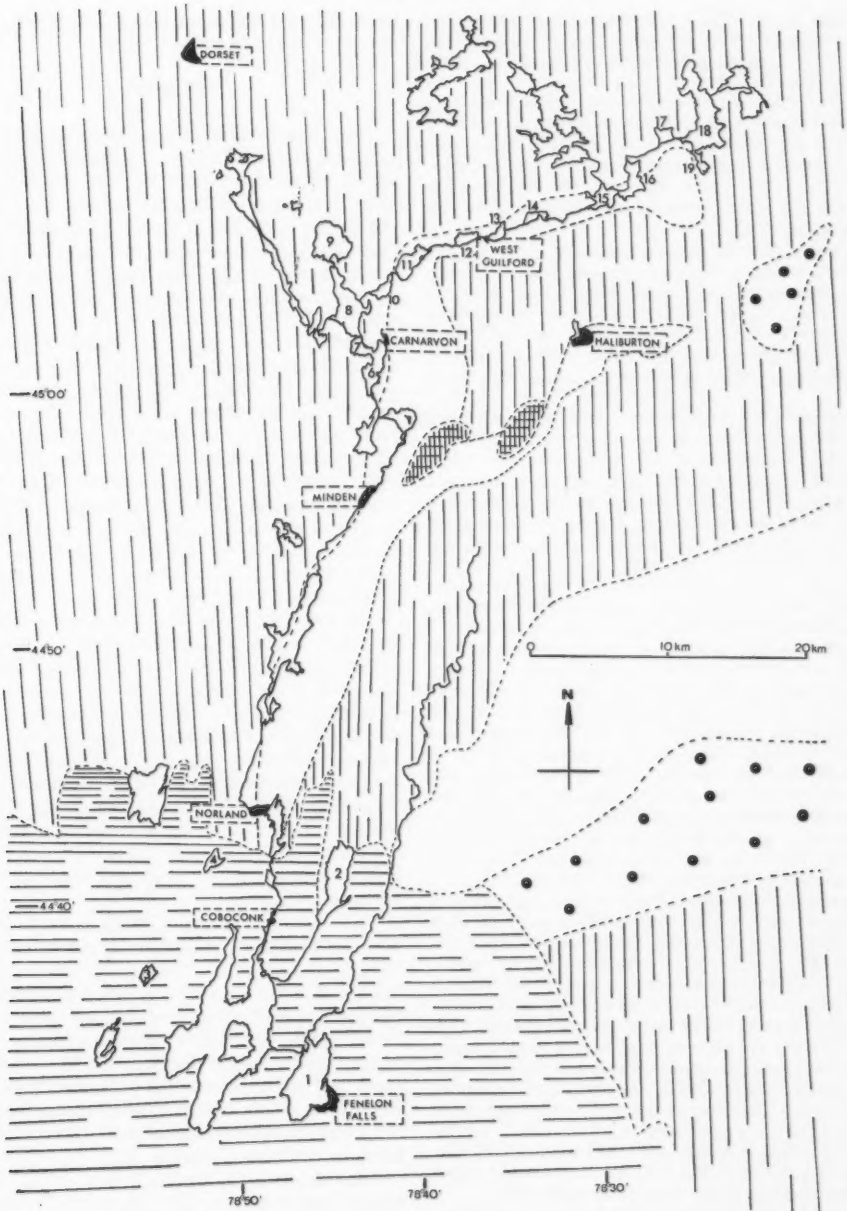

FIG. 1. Physiography of the study region and location of the lakes: vertical lines, Precambrian Grenville elastic metasediments; horizontal lines, Paleozoic Ordovician limestone, dolomite; cross-hatching, Precambrian Grenville igneous rocks; ●, Precambrian sediments, quartzite, schist, conglomerate; white, Precambrian Grenville carbonate metasediments. (Lakes: 1, Cameron; 2, Four Mile; 3, Raven; 4, Talbot; 5, Bob; 6–8, Twelve Mile–Boshkung; 9, Halls; 10, Beech; 11, Maple; 12, Green; 13, Pine; 14, Cranberry; 15–16, Eagle–Moose; 17–19, Oblong–Haliburton.)

1–3 times weekly. For a few streams that were particularly inaccessible or unsuitable for any of the techniques for measuring discharge, we used the mean monthly runoff figures given by Pentland (1968) and Coulson (1967). Measurements were made from May 1971 to December 1972. Although measurements were less frequent and perhaps less reliable in 1971, we have calculated water and nutrient budgets for two overlapping, 12-mo periods, the first from May 1971 to May 1972 and the second from January 1972 until the end of December 1972.

Water samples for analysis of total P in large rivers were taken with a Van Dorn bottle lowered from a bridge, and in smaller streams with a wide-mouthed, polyethylene bottle. Subsamples were immediately transferred to the Erlenmeyer flasks in which they would eventually be digested and analyzed for total P. All flasks were sealed before and after use with Parafilm.

Samples of lake water were collected twice from each lake within 5 days of ice melt in May 1972. Water from three stations in all but the two smallest lakes, which were sampled at two sites, was collected using a weighted, 19 mm ID Tygon tube that was lowered to within 1 m of the lake bottom, clamped at water level, raised by a nylon line attached to the bottom end, and emptied through a 216-μ screen into a polyethylene bucket. Triplicate, 50-ml subsamples from the pooled samples were immediately transferred to Erlenmeyer flasks. Particulate material >216 μ was collected in a tow net lowered to within 2 m of the bottom and hauled vertically to the surface. Duplicate tows were collected and no correction was applied for efficiency of the net. Originally we intended to add particulate P >216 μ to get total P, but our analyses showed that because zooplankton populations are low at the time of spring overturn, less than 5% of the total P was normally in particles >216 μ. Because zooplankton are patchily distributed and comprise a small fraction of the total, we decided to exclude them from our analyses. Therefore, our estimate of total P in lake water at spring overturn is really total P <216 μ.

To sample precipitation, four standard polycarbonate rain gauges were set up in the study area. They were left continuously uncovered to collect dry fallout as well as rain, but to reduce contamination by insects, a 76-μ nylon net was attached to the funnel bottom. Snow samples were collected in a polyethylene basin and total P analyses of snow were combined with snowfall data collected by the nearest meterological stations to give phosphorus loading from snowfall.

All glassware was carefully washed, first by rinsing with tap water and then by soaking for 24 h in saturated NaOH to remove organic contamination. After a second tap water rinse, it was rinsed in concentrated HNO_3, soaked in 2–5% HNO_3 or HCl for 24 h, rinsed 6 times with deionized, distilled water, and oven dried.

Chemical analyses for total P were done in triplicate by the method of Parsons and Strickland (1968) with the following minor modifications. All samples were digested to dryness in 125-ml Erlenmeyer flasks. Then after addition of 50 ml of 0.1% HCl, they were left for 48 h before addition of reagents. Ten milliliters of the acid–molybdate–potassium antimony tartrate mixture were added to a 50-ml sample.

Results

The location and general characteristics of the lakes are given in Table 1. The catchment area (A_d) varied from only 14 km^2 for Talbot Lake to 3250 km^2 for Cameron Lake, but because the lakes with the largest A_d also had the largest surface area (A), the range in the ratio of A_d to A was only 5.8–390. Mean depth of our lakes ranged from 0.7 to 27 m. Although lakes with a much greater mean depth are to be found in many of the world's lake districts, they are rare in the region we studied. The conductivity data show that the lakes fall into three categories depending on their physiography. The lakes in granite had the lowest conductivity, those in Precambrian areas with some carbonate sediments had intermediate conductivity, and those with Ordovician limestone in their catchment area had the highest. Comparison of these values and the hardness with the results of extensive lake surveys in southern Ontario (Ryder 1964; Sparling and Nalewajko 1970) showed that our lakes are typical of the regions in which they occur.

The hydrologic and total phosphorus budgets of the lakes are summarized in Table 2. Since data collecting began in May 1971 and ended in December 1972, it was necessary to allow a 4-mo overlap to obtain two 12-mo budgets. Obviously there was a considerable difference between years, probably because 1972 was a year of above average precipitation and therefore runoff. The areal runoff (m yr^{-1}) was about 25% higher in 1972 than the long-term average (Pentland 1968). This factor accounts for the increased flushing rates of all lakes in 1972 and is also reflected in the increased loadings of most lakes in 1972.

The variation in the flushing rates is 300-fold, from as low as 0.21 yr^{-1} for Four Mile Lake in 1971 to as high as 62.7 yr^{-1} for Cranberry Lake in 1972, the latter figure being the equivalent of replacing the lake volume more than once a week. These lakes differ from Vollenweider's (Vollenweider 1969) lakes in that 9 of the 13 have flushing rates $>$ 1 yr^{-1}, whereas the maximum rate for the Swiss lakes was 0.68 yr^{-1}. The figures for these lakes are, however, fairly typical of most of the recreational lakes in southern Ontario.

The phosphorus loadings also cover a wide range, from a high of 2210 mg m^{-2} yr^{-1} (Cameron Lake, 1972) to a low of 98 mg m^{-2} yr^{-1} (Talbot Lake, 1972). Four of the lakes have annual loadings of $>$1000 mg m^{-2} yr^{-1}, with the highest, Cameron Lake, being about double that of Lake Erie (International Joint Commission 1969; Patalas 1972).

TABLE 1. Location, physiography (L, limestone plain; T, till plain; S, sand plain; PG, Precambrian granite; PS, Precambrian carbonate sediments), land use (MF, mixed forest; R, recreational; B, bogland; P, pasture; F, agriculture [fertilized]), morphometry (A, lake surface area; A_d, drainage area; $\bar{z}$, mean depth; V, lake volume), and conductivity and hardness at spring overturn in 13 southern Ontario lakes.

Lake	No.	N. lat.	W. long.	Physiography	Land use	A (km^2)	A_d (km^2)	$\bar{z}$ (m)	V ($\times 10^6 m^3$)	Conductivity ($\mu mho\ cm^{-3}$)	Hardness (ppm)
Cameron	1	44°33′	78°46′	L,T,S	MF,R,F,P	12.72	3250	7.1	89.9	122	60
Four Mile	2	44°41′	78°45′	L,PG	MF,B,R	7.73	45	9.3	71.4	200	104
Raven	3	44°37′	78°55′	L	B,MF	1.07	45	0.73	0.78	287	160
Talbot	4	44°42′	78°51′	L	MF,B,R	1.07	14	0.85	0.91	–	–
Bob	5	44°54′	78°47′	PG	MF,R	2.27	29	18.0	40.7	46	19
Twelve Mile–Boshkung	6	45°02′	78°44′	PG	MF,R	11.65	933	18.1	214.0	52	22
Halls	7	45°06′	78°45′	PG	MF,R	5.32	270	27.2	145.0	37	13
Beech	8	45°05′	78°42′	PG	MF,P,R	1.36	515	9.8	13.3	59	29
Maple	9	45°06′	78°40′	PG,PS	MF,P,R	3.32	502	11.6	38.6	60	22
Pine	10	45°07′	78°35′	PG,PS	MF,P,R	1.08	285	7.4	8.05	75	21
Cranberry	11	45°07′	78°34′	PG,PS	MF,P,R	0.69	269	3.5	2.44	65	27
Eagle–Moose	12	45°09′	78°28′	PG,PS	MF,R	5.15	251	12.8	65.8	64	25
Oblong–Haliburton	13	45°11′	78°25′	PG	MF,R	11.03	143	17.7	196.0	52	25

TABLE 2. The flushing rate (ρ), total phosphorus loading rate (L), and total phosphorus retention coefficient (R) for 13 southern Ontario lakes.

Lake	$\rho(yr^{-1})$		L($mg\ m^{-2}\ yr^{-1}$)		R	
	1971	1972	1971	1972	1971	1972
Cameron	13.8	18.9	1700	2210	0.38	0.30
Four Mile	0.21	0.26	108	107	0.85	0.82
Raven	12.2	14.9	200	217	0.56	0.55
Talbot	–	4.9	–	98	–	0.69
Bob	0.30	0.37	153	162	0.75	0.71
Twelve Mile–Boshkung	1.87	2.38	311	352	0.40	0.33
Halls	0.83	0.96	194	217	0.53	0.53
Beech	16.8	22.7	1360	1680	0.05	0.07
Maple	5.9	8.0	803	860	0.37	0.26
Pine	16.3	18.5	1050	1060	0.04	0.01
Cranberry	55.7	62.7	1320	1280	–0.02[a]	–0.07[a]
Eagle–Moose	1.82	2.03	217	232	0.27	0.36
Oblong–Haliburton	–	0.32	–	124	–	0.72

[a]Although it is possible to have a negative retention coefficient on a short-term basis, because of loss of materials from the sediments, these figures more likely reflect the experimental error associated with these budget calculations.

Despite these wide variations in loading and flushing rate, a comparison of the predicted total phosphorus concentrations, calculated as described previously, and the measured total phosphorus concentrations shows that for almost every lake the two values are close. Theoretically the best comparison should be between the lake concentrations measured in the spring of 1972 and the values predicted from the nutrient budget of May 1971–April 1972. The budget data of January 1972–December 1972, however, were more accurately measured, and since the steady state solution to the model was employed, a comparison between these budgets and the spring 1972 measured value is valid (See Table 3).

The only exception to a good fit is Talbot Lake, with a measured concentration of total phosphorus of 15.3 mg m^{-3}, well above the predicted value of 7.3. This is interesting because Talbot Lake, being sampled only once, was the least thoroughly studied, since all other lakes were sampled twice at spring overturn. Also, the phosphorus budget calculation for Talbot Lake was the least precise because this lake alone had no stream inflows. Phosphorus loading was calculated from an estimation of the volume of seepage from the lake's small drainage area, a small number of analyses of the phosphorus concentrations in this ground water, and the calculated load from precipitation. Furthermore, Talbot Lake is extremely shallow ($\bar{z} < 1$ m) as is Raven Lake, and as will be shown below, the correlations are much better if both are excluded.

Correlations of the measured and predicted values with and without Talbot and Raven lakes included are given in Table 4. The best correlation coefficient for the southern Ontario lakes alone ($r = 0.90$) was found between the 1972 measured concentration

TABLE 3. Measured [P] and predicted ($L(1-R)/\bar{z}\rho$) total phosphorus concentration at spring overturn in 13 southern Ontario lakes.

Lake	[P] ($mg\ m^{-3}$)	$L(1-R)/\bar{z}\rho$ ($mg\ m^{-3}$)	
	1972	1972[a]	1973[b]
Cameron	12.4	10.8	11.5
Four Mile	12.0	8.3	8.0
Raven	8.3	9.9	9.0
Talbot	15.3	–	7.3
Bob	8.5	7.1	7.0
Twelve Mile–Boshkung	6.1	5.4	5.4
Halls	4.3	4.0	3.9
Beech	8.5	7.8	7.0
Maple	9.3	7.4	6.8
Pine	10.5	8.4	7.7
Cranberry	9.9	6.9	6.2
Eagle–Moose	7.1	6.8	5.7
Oblong–Haliburton	5.3	–	6.1

[a]Budget measured from May 1971–April 1972.
[b]Budget measured from January 1972–December 1972.

TABLE 4. Results of correlation analysis between measured (Y) and predicted (X) total phosphorus concentrations for southern Ontario lakes with and without additional Swiss lakes.

Y ([P] measured)	X ($L(1-R)/\bar{z}\rho$)	n	Lakes left out	r
Overturn, 1972	May 1971–Apr. 1972[a]	11	Talbot, Raven	0.90
"	"	13	–	0.62
"	Jan. 1972–Dec. 1972	11	Talbot, Raven	0.85
"	"	13	–	0.65
" + four Swiss lakes[b]	May 1971–Apr. 1972[a] (Ontario lakes)	15	Talbot, Raven	0.97
"	"	17	–	0.95
"	Jan. 1972–Dec. 1972[a] (Ontario lakes)	15	Talbot, Raven	0.97
"	"	17	–	0.96

[a]Including January 1972–December 1972 data for lakes with only one 12-mo period.
[b]From Vollenweider (1969).

and the predicted concentration from the first 12-mo period, with Talbot and Raven Lake excluded. With the two shallow lakes included, the correlation dropped to 0.62. Additional information allowing similar calculations was given by Vollenweider (1969) for a number of Swiss lakes. For the four lakes that could be classed as oligotrophic or mesotrophic, the phosphorus concentration at spring overturn was very similar to the value predicted from $L(1-R)/\bar{z}\,\rho$; 7.6 vs. 9.1 µg/liter for the Aegerisee, 14.5 vs. 9.2 µg/liter for the Türlersee, 40 vs. 48.4 µg/liter for the Hallwilersee, and 32 vs. 29.1 µg/liter for the Zürichsee–Urtersee. For the remaining four which were eutrophic lakes, the measured and predicted values were not in close agreement (115 vs. 69.4 µg/liter for the Bodensee, 88 vs. 45.1 µg/liter for the Pfäffikersee, 118 vs. 63.9 µg/liter for the Greifensee, and 162 vs. 91.2 µg/liter for the Baldeggersee). The correlation coefficient between measured and predicted concentrations when 11 southern Ontario and four Swiss lakes are combined is 0.97; with 13 southern Ontario lakes and four Swiss lakes, it is 0.95. Results were almost identical when the second 12-mo period for nutrient budget calculations was used.

Discussion

The results of these studies have shown that the total phosphorus concentration at spring overturn in oligo- and mesotrophic lakes can be reasonably accurately predicted from the total phosphorus loading (L), mean depth ($\bar{z}$), water flushing rate (ρ), and phosphorus retention coefficient (R) over a broad range of loading and flushing rates. More studies are needed, however, in regions with higher nutrient concentrations and in shallower and perhaps smaller lakes.

The importance of this simple model lies in the fact that it can be combined with other models relating phosphorus concentration to water quality parameters. A good example of this is the spring phosphorus concentration–summer chlorophyll *a* concentration model first described by Sakamoto (1966) and since expanded by Dillon (1974). This simple empirical relationship predicts the average chlorophyll *a* concentration in summer in a lake from a measurement of the total phosphorus concentration at spring overturn. For a given change in phosphorus concentration, the corresponding change in chlorophyll *a* concentration can be estimated. The application of the Vollenweider-type model as a means of predicting the change in phosphorus concentration from a given change in phosphorus loading obviously will make the phosphorus–chlorophyll model much more useful.

The main difficulty with the phosphorus concentration model is, at present, that it is much more difficult to measure the nutrient and water budgets of a lake than it is to measure the spring phosphorus concentration. The real value of the model will be realized when it is possible to predict, rather than measure, L, ρ, and R. The flushing rate, ρ, can be calculated reasonably well from meteorologic and hydrologic parameters (rainfall, evapo-transpiration, runoff) and from the lake's morphometry (volume). Phosphorus loading can be estimated from consideration of export from soils, per capita discharge of sewage, etc., as done by Patalas (1972), Patalas and Salki (1973), Shannon and Brezonik (1972), and the Nutrient Sources Subcommittee (1966), although, in general, more reliable means of making estimates are needed. We still lack a quantitative means of predicting R for a given lake.

When all these requirements are met, we will have the basis for a simple scheme requiring a

minimal field program with respect to the problem of eutrophication.

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Primary Productivity of the Benthic Microalgae in the Chukchi Sea near Barrow, Alaska[1]

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MATHEKE, G. E. M., AND R. HORNER. 1974. Primary productivity of the benthic microalgae in the Chukchi Sea near Barrow, Alaska. J. Fish. Res. Board Can. 31: 1779–1786.

The primary productivity of the benthic microflora was measured using in situ techniques. Primary productivity ranged from below 0.5 mg C m^{-2} h^{-1} in winter when the sampling area was covered with ice to nearly 57.0 mg C m^{-2} h^{-1} in August. This is 8 times the productivity of the ice algae and twice that of the phytoplankton.

MATHEKE, G. E. M., AND R. HORNER. 1974. Primary productivity of the benthic microalgae in the Chukchi Sea near Barrow, Alaska. J. Fish. Res. Board Can. 31: 1779–1786.

La production primaire de la communauté algale benthique a été mesurée à l'aide de techniques in situ. Les taux varient de moins de 0.5 mg C m^{-2} h^{-1} en hiver sous la glace à près de 57.0 mg C m^{-2} h^{-1} en août, soit huit fois la production des algues dans la glace et deux fois celle du phytoplancton.

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THE primary productivity of the benthic microalgae has received increased attention in recent years (Pomeroy 1959; Grøntved 1960, 1962; Wetzel 1964; Steele and Baird 1968; Hargrave 1969; Gargas 1970, 1972; Hickman and Round 1970; Leach 1970; Bunt et al. 1972). Benthic microalgae often account for a significant percentage of the total primary productivity of estuarine and inshore ecosystems. Our objectives were to assess the primary productivity of the benthic microalgae in a nearshore area of the Chukchi Sea and to compare it with the primary productivity of the phytoplankton and sea ice algae in this ecosystem.

Materials and Methods

In situ techniques, utilizing SCUBA, were employed for primary productivity measurements of the benthic microalgae and ice algae. Water samples containing phytoplankton were also incubated in situ. In situ methods minimized physical disturbance of the communities and prevented exposure of the algae to surface light and temperature.

Incubation chambers for measurement of benthic productivity were plastic cylinders (3.4 cm ID) closed at one end with a plexiglass plate. A hole in the plate accepted a small rubber stopper, and a sidearm equipped with a rubber sleeve-type serum bottle stopper was placed near the top of the cylinder. The bottom of the cylinder was beveled on the outside to minimize disturbance of the sediment during penetration. Once the chamber was positioned, a rubber stopper was inserted in the top plate and 5 μCi of $NaH^{14}CO_3$ were injected through the serum cap with a hypodermic syringe. The syringe was pumped several times to insure adequate mixing and the chamber was pressed further into the sediment to insure penetration of the label into the sediment. Leach (1970) reported that the label was detected in the 1–2-mm layer when incubation chambers were treated in this manner.

Following breakup of the shorefast ice that covered the study area until mid-July, an algal mat of the filamentous diatom *Amphipleura rutilans* (Trent.) Cleve developed. Because it was impossible to use the plexiglass incubation chambers while this layer was present, a new apparatus was devised. A sharpened metal spatula was inserted under the algal mat and small cores were cut with a cork borer (1.6 cm ID) using the spatula as a base to facilitate cutting the filaments. These cores were transferred to 250-ml squat jars and covered with bakelite caps fitted with serum bottle stoppers. Five μCi $NaH^{14}CO_3$ were injected through the serum cap, and the jars were inverted slowly and placed in a plexiglass holding box

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that was left lying on the sediment during the incubation period. When the jars were inverted, the core settled on the cap with the filament side up because the dense layer of compacted mud present beneath the algal mat inverted the core as it fell.

One dark and three light incubation chambers were used for each determination. Samples were incubated for 5 h between 0900 and 1400. After incubation the algae were preserved with one drop of concentrated H_3PO_4 and transported to the laboratory. The supernatant water was drawn off and the top centimeter of each core was transferred to a 50-ml centrifuge tube. The sample was washed twice with 0.005 N HCl and centrifuged for 10 min at 2000 × *g* after each washing. The sample was then transferred to a combustion vial and frozen until combustion could be carried out.

Sediment samples were prepared for counting following the method developed by Stanley (1971). Samples were oxidized by wet combustion in a boiling sulfuric acid–potassium dichromate mixture. The evolved carbon dioxide was trapped in a liquid scintillation cocktail containing phenethylamine. Recovery of ^{14}C-labelled glucose mixed with sediment was 94.9% (90% confidence interval = 1.79). Samples were counted in a Nuclear Chicago Model 6848 liquid scintillation counter and corrected for quenching by the external standard method. Total CO_2 was determined by analyzing a water sample taken just above the sediment (Strickland and Parsons 1968).

The method of determining the primary productivity of ice algae has been described by Clasby et al. (1973). Primary productivity of the phytoplankton was determined by adapting the Steemann Nielsen (1952) technique to liquid scintillation counting. Wet filters were placed in the liquid scintillation cocktail (Aquasol) and counted in a Nuclear Chicago Model 6848 liquid scintillation counter; counting efficiency was determined by the channels ratio method. Seawater primary productivity samples were incubated in situ in plexiglass incubation tubes suspended beneath the ice until breakup of the ice (Horner and Alexander 1972a). After this time they were suspended at various depths from a line attached to a surface buoy.

Sediment samples for chlorophyll *a* analysis were taken with plexiglass core tubes (4.8 cm ID). Two replicate cores were taken from each station. The samples were returned to the laboratory, the supernatant water was drawn off, and 1-cm sections were cut from each of the upper 4 cm, placed in petri dishes, and frozen. Before analysis, the samples were thawed in the dark at room temperature; each sample was well mixed and two subsamples were taken from each core. The subsamples were added to a mortar containing 0.1 g of $MgCO_3$, ground for 1 min with 10 ml 90% acetone, and washed into a centrifuge tube; then the volume was brought up to 20 ml. They were extracted in the dark at 5 C for 16 h and then centrifuged for 10 min. Chlorophyll *a* was determined in a Beckman DU-2 spectrophotometer by the method of Lorenzen (1967). Lorenzen's (1967) equations were modified to express concentrations on a m^{-2} basis.

Core samples from each station were examined with a microscope to determine relative abundance of the algal species present in the benthic and ice communities; net tows and water bottle samples were examined to determine species present in the phytoplankton. Light was measured with a Sekonic underwater light meter and results were compared with a Gossen Super Pilot light meter for conversion to lux.

While the study area was covered by shorefast ice, only one site was occupied. It was located about 1.0 km offshore from the Naval Arctic Research Laboratory, Barrow, Alaska (71° 20′ N, 156° 41′ W). Water depth at this site was about 5 m and the sediment was composed of mud (silt–clay) mixed with fine and very fine sands based on the Wentworth scale (Wentworth 1922). Following breakup of the ice in mid-July, site 2 was established about 0.1 km offshore. The depth and sediment composition at this site were similar to site 1.

Results

Until July, while the ice cover was still present, the sediment community was composed primarily of motile, unattached diatoms (Table 1). Small patches (3–5 cm^{-2}) of the filamentous diatom *A. rutilans* were first noticed on June 22, a few days before sampling was discontinued due to the weakness of the ice. By July 12, after the ice had broken up, a mat of this diatom covered the sediment. This layer was still present when sampling was discontinued on August 23. Unattached diatoms were present both among the filaments and in the sediments below the *A. rutilans* mat. *Licmophora ehrenbergii* (Kuetz.) Grunow and a species resembling *L. oedipus* (Kuetz.) Grunow were common as epiphytes on *A. rutilans*.

Chlorophyll *a* concentrations in the top centimeter ranged from 35 to 112 mg m^{-2} from February until late May (Fig. 1). Chlorophyll *a* concentrations increased slightly in June, reaching 138 mg m^{-2} by June 26. On July 20, after the *A. rutilans* mat developed, the chlorophyll *a* concentration at site 1 had reached 251 mg m^{-2}. During July and August chlorophyll *a* concentrations at site 1 ranged from 250 to 321 mg m^{-2}. Chlorophyll *a* concentrations were lower at site 2 except on 10 August. An analysis of variance indicated that there was a significant change in chlorophyll concentration with depth in the sample core ($P < 0.05$). The only significant temporal change in chlorophyll concentration occurred in the samples from the upper centimeter ($P < 0.05$).

The primary productivity of the ice algae, phytoplankton, and benthic microalgae is shown in Fig. 2. During May a bloom occurred in the ice and primary productivity reached a maximum of 4.6 mg C m^{-2} h^{-1} on May 21 (Clasby et al. 1973). Subsequently it declined rapidly and the community disappeared

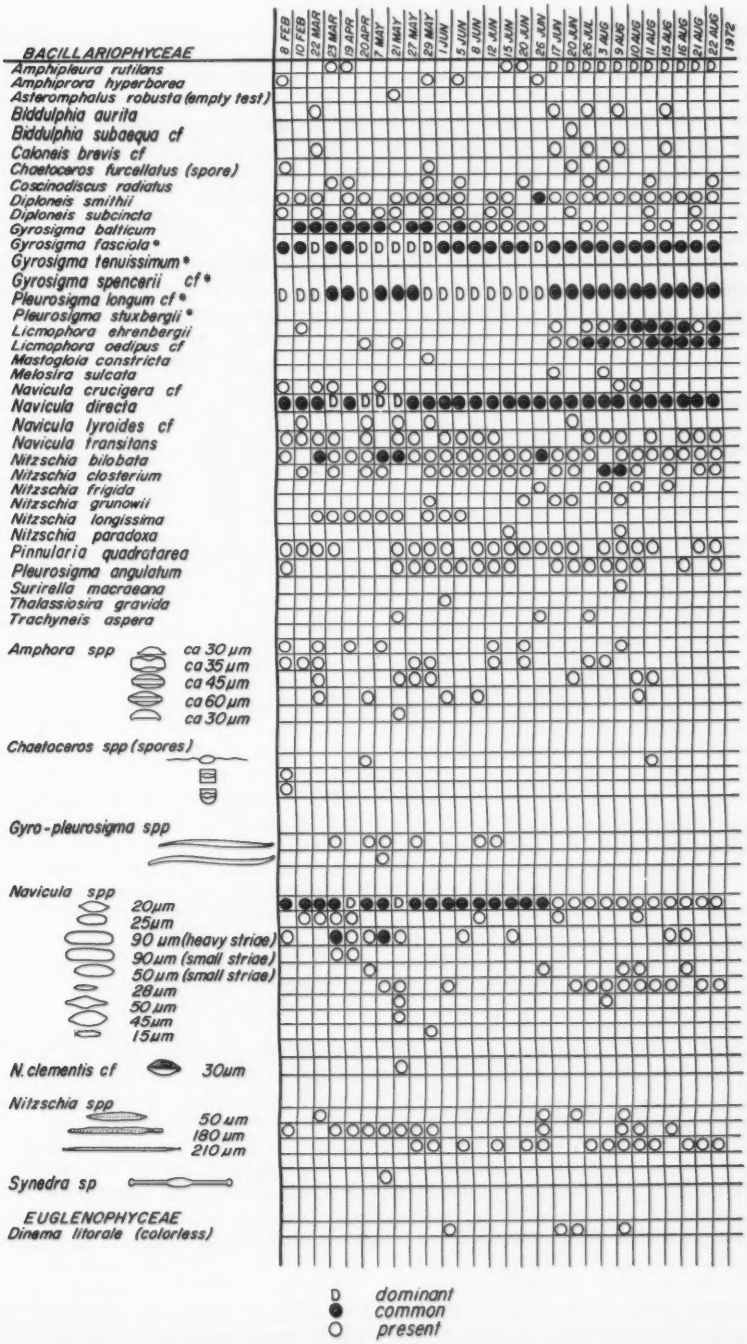

TABLE 1. Benthic microflora at Barrow, Alaska, February–August 1972. *Several species that could not be identified without clearing the frustules were not separated in routine enumeration.

about June 10. Primary productivity values for the benthic community were less than 0.5 mg C m^{-2} h^{-1} from February until mid-June. Following the disappearance of the ice community, productivity of the benthic microalgae increased to 1.9 mg C m^{-2} h^{-1} by the end of June and to 20.7 mg C m^{-2} h^{-1} by mid-July. Much of the increase was due to the growth of the *A. rutilans* mat. The highest primary productivity at site 2 ranged from 2.4 to 33.0 mg C m^{-2} h^{-1} and was always lower than at site 1. A depression in the productivity at site 2 on August 10 and at site 1 on August 11 occurred following a severe storm that deposited about a centimeter of sediment on the *A. rutilans* mat. Productivity of the water under the ice from February through the time of the ice algal bloom ranged from 0.0 to 0.6 mg C m^{-2} h^{-1}. During the summer the highest phytoplankton productivity was 17.6 mg C m^{-2} h^{-1} at site 1 on August 11 and 23.8 mg C m^{-2} h^{-1} at site 2 on August 22.

Discussion

The development of a 2–4 cm thick bloom in the underside of the ice during late April–May is a regular occurrence. It is apparently triggered

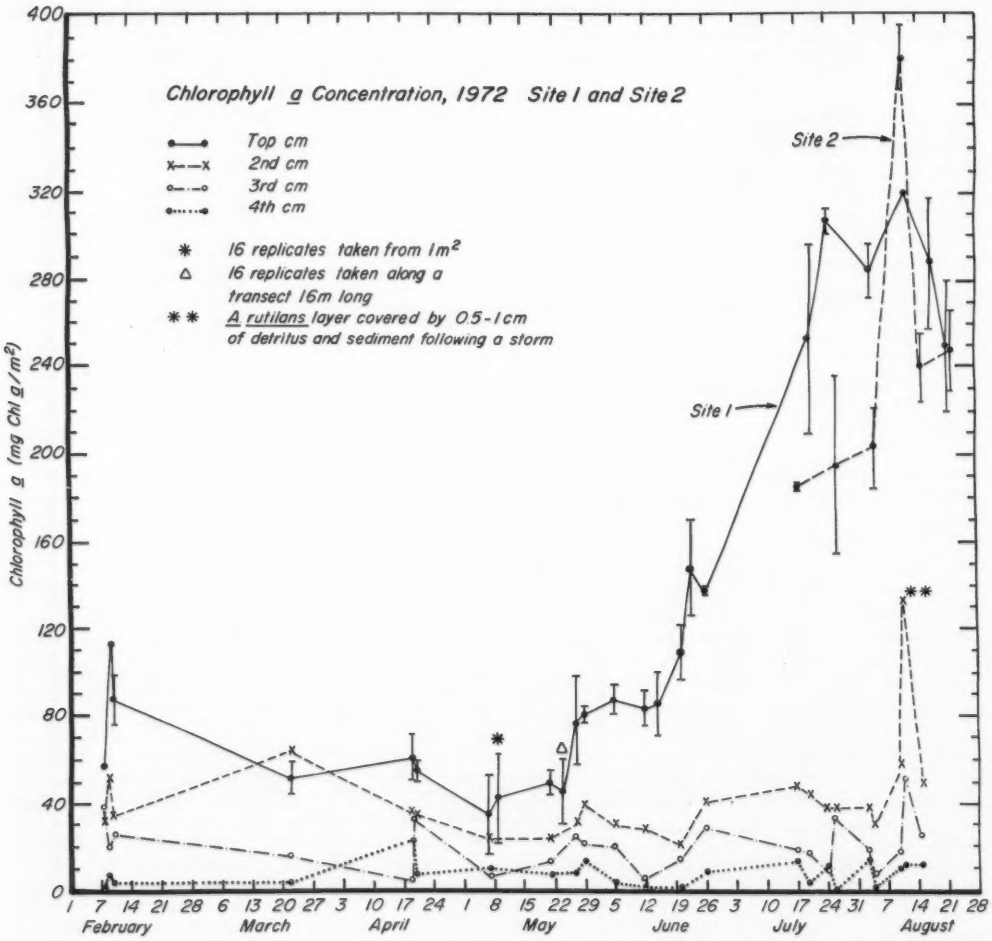

FIG. 1. Chlorophyll *a* concentration of the upper 4 cm of sediment February–August 1972. Vertical bars represent the standard deviation. Data for site 1 and site 2 were pooled in the second through fourth cm.

by increasing light levels due to increased solar radiation and dissipation of snow cover. The bloom ends when the ice begins to melt and extensive brine drainage occurs. Productivity of the benthic microalgae increases when the ice algal bloom disappears. This increase coincides with the formation of melt ponds on the ice surface which, along with the disappearance of the ice algae, increases light transmission through the ice. Light penetration through the ice increased from 0.8 to 16.7% of the incident intensity (in lux) during this period (May 7–June 26 1972).

The ice is an inverted benthic habitat and many of the organisms living in the ice, especially the diatoms, behave like benthic organisms. We therefore thought that some of the ice algae might sink to the bottom and continue to grow, thus increasing the productivity of the benthos. Indeed, many of the species inhabiting the sediment (Table 1) are also present in the ice (Horner and Alexander 1972b). However, some of the species found in the ice, including several of the most abundant, are not found in the sediment either during or after the bloom in the ice. A more detailed taxonomic study of both communities is necessary, but at present there is little evidence to suggest that this is the fate of the ice organisms. Increases in productivity and chlorophyll *a* concentrations in the sediment following the bloom in the ice can probably be ascribed to coincident increases in light penetration through the ice.

The benthic microalgae became the most important source of primary productivity in this nearshore ecosystem after the breakup of the shorefast ice and the development of the *A. rutilans* mat. Primary productivity of the benthic microalgae at site 1 was 8 times that of the ice algae and twice that of the phytoplankton. If the productivity of the benthic microflora in the areas studied is not a local phenomenon, the benthic community would represent a significant source of primary productivity in this ecosystem. At present, we can only speculate on the utilization of these algae by higher trophic levels.

Although the methods employed to measure primary productivity in various studies differ, several comparisons are of interest. The absence of data for the period from September to February precludes accurate calculation of annual mean productivity for the benthic microalgae. However, assuming that productivity is negligible from the time the shorefast ice is usually formed in late September until February, the annual mean productivity would probably be less than 5 mg C m^{-2} h^{-1}. The summer maxima at Barrow (57 mg C m^{-2} h^{-1}, site 1; 33 mg C m^{-2} h^{-1}, site 2) are about an order of magnitude less than those reported by Grøntved (1960, 1962) and Gargas (1970) for shallow waters (<1 m) and intertidal areas in Denmark. The annual mean productivities found by these authors ranged from 31 to 68 mg C m^{-2} h^{-1}. Bunt et al. (1972) reported a mean productivity of 8.1 mg C m^{-2} h^{-1} and a maximum of about 14 mg C m^{-2} h^{-1} for samples of sediment in the Carribean Sea taken mostly from 10 to 16 m. Even though the productivity during the ice-free period at Barrow is much greater than the maximum in the Caribbean, the mean productivities are similar due to the shorter growing season. In a shallow tundra pond near Barrow, Stanley (1973) recorded productivity values for the benthic microflora that ranged from 4 to 30 mg C m^{-2} h^{-1} during the period of thaw (June–August).

A possible source of error in the measurement of benthic productivity is the assumption that the algae photosynthesize only at the surface of the sediment and that they obtain all of the CO_2 for photosynthesis from the water column. It is quite probable that the algae can utilize CO_2 present in the interstitial water as well as in the water column (Bunt et al. 1972). If the $NaH^{14}CO_3$ added to the incubation chambers does indeed penetrate to the second millimeter of sediment without lowering the specific activity, this should not introduce a significant error; the rapid attenuation of light by the sediment (Fenchel and Straarup 1972) would prevent photosynthesis below this level. The use of samples taken from the water immediately above the sediment for determination of total CO_2 may introduce a more serious error. The total CO_2 in the interstitial water (36.0 ± 4.8 mg liter^{-1}) was significantly greater than that found in the water column (25.1 ± 3.0 mg liter^{-1}). If the algae are capable of utilizing CO_2 from the interstitial water our productivity figures would be underestimates.

The existence of small-scale patchiness in the distribution of the benthic microalgae is suggested by the large standard deviations of replicate cores (taken within 5 cm of each other) for primary productivity and chlorophyll *a* determinations (Fig. 1 and 2). The presence of brown patches of epipelic algae noted by divers during June corroborates this evidence. The brown patches (9–18 cm^{-2}) had chlorophyll *a* concentrations from 2 to 4 times those of the surrounding sediment. Several other investigators (Grøntved 1960, 1962; Fenchel and Straarup 1971; Bunt et al. 1972) have reported the presence of small-scale patchiness in the distribution of benthic microalgae. Grøntved (1960) suggested that growth was more rapid in less disturbed areas where finer sediments accumulated, and therefore, patchiness might be related to the mud content of the sediment. In this study divers observed that the brown patches of epipelic algae were usually

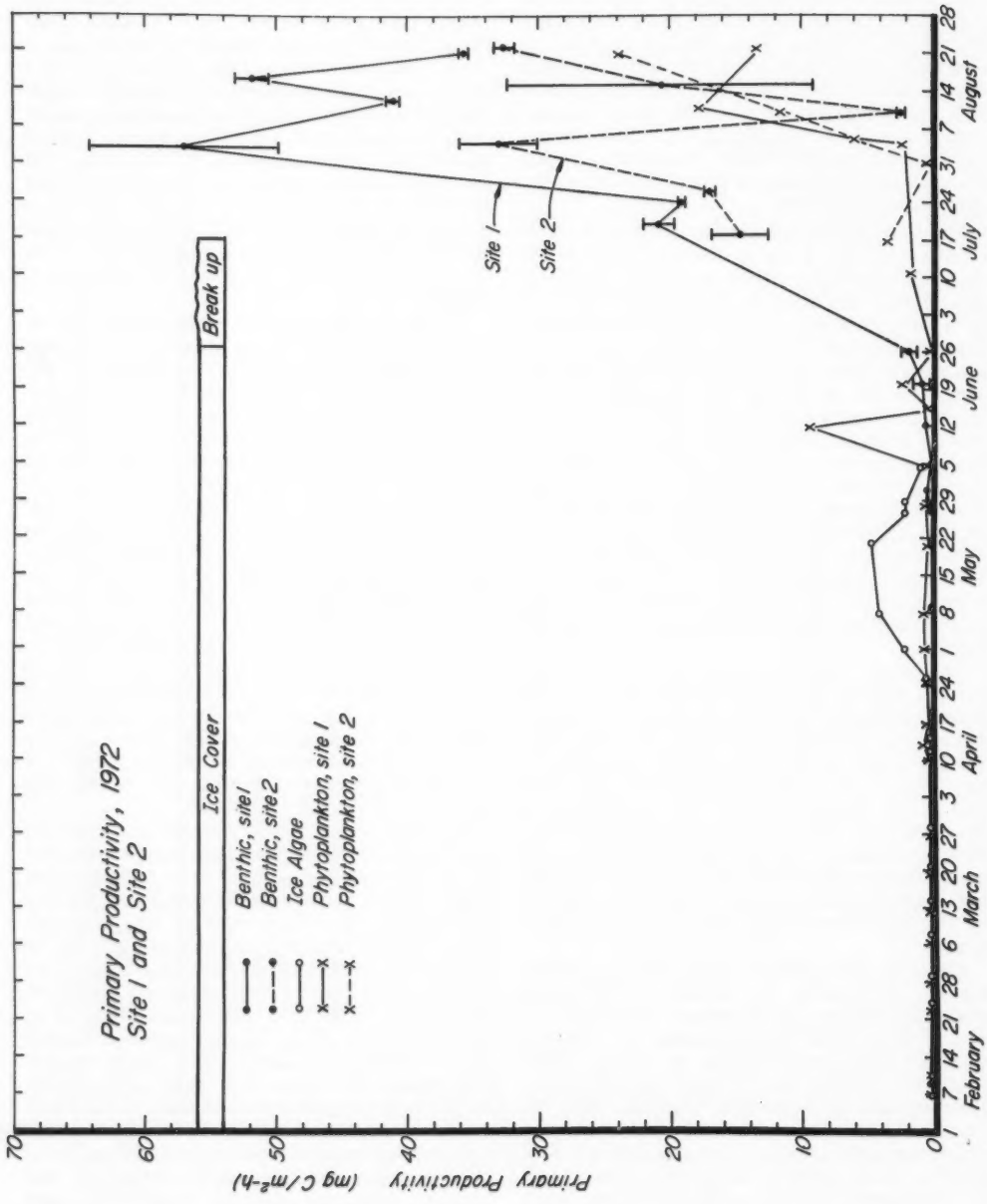

FIG. 2. Primary productivity of the ice algae, phytoplankton, and benthic microalgae. Vertical bars represent the standard deviation.

found in troughs where the mud content would be expected to be highest. However, linear regression analyses showed there was no significant correlation between the silt-clay (mud) and the chlorophyll *a* content of cores ($P > 0.05$).

Chlorophyll *a* concentrations were relatively high during the winter months when the benthic microalgae were subjected to a long period of darkness and primary productivity was negligible. In addition, chlorophyll *a* levels remained more or less constant in the second, third, and fourth cm of sediment throughout the sampling period. Microscopic examination of samples taken from the lower levels of sediment showed that many of the cells were healthy and often contained reserve food material. These observations were also true of the upper centimeter of sediment during February and March. Thus, a substantial number of algae appear to spend much of their time out of the photic zone. Previous investigations of the benthic microalgae have shown the presence of pigmented cells well below the photic zone (Grøntved 1962; Steele and Baird 1968; Meadows and Anderson 1968; Fenchel and Straarup 1971). Horner and Alexander (1972b) and Rodhe (1955) reported the presence of viable cells in the darkness in ice-covered ecosystems. Heterotrophic uptake of dissolved organic substrates has been suggested as an energy source for photosynthetic organisms in environments where long periods of darkness occur (Wilce 1967; Wood 1956). As a result of investigations in Loch Ewe, Scotland, by Steele and Baird (1968), Munro and Brock (1968) investigated the heterotrophic potential of the sand community there. They found that bacteria alone were responsible for heterotrophic uptake and that algal heterotrophy was negligible in this system. These observations agree with the findings of Horner and Alexander (1972b) for the ice algal community at Barrow.

Several investigators (Curl and McLeod 1961; Antia and Cheng 1970; Bunt and Lee 1972) have offered another solution to this problem by demonstrating that axenic cultures of diatoms are capable of surviving in the darkness for periods of up to 2 mo. Additional study is required to determine how the benthic microalgae at Barrow are able to survive long periods of darkness. This should include an investigation of heterotrophic uptake by this community as well as attempts to maintain axenic cultures of the major species in the dark for extended periods of time.

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NOTES

Effects of Thyroid Hormones on Behavior of Yearling Atlantic Salmon (*Salmo salar*)

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GODIN, J.-G., P. A. DILL, AND D. E. DRURY. 1974. Effects of thyroid hormones on behavior of yearling Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 31: 1787–1790.

Swimming activity, aggressive behavior, and upstream orientation of yearling Atlantic salmon (*Salmo salar*) treated with 6.43×10^{-11} M thyroxine were significantly lower than those of control fish injected with solvent alone. Two concentrations of triiodothyronine (7.43×10^{-11} M; 7.43×10^{-10} M) caused similar but less pronounced effects.

Because similar behavioral modifications accompany smolt migration, we hypothesize that thyroid hormones may play a role in arousing migratory tendencies in Atlantic salmon.

GODIN, J.-G., P. A. DILL, AND D. E. DRURY. 1974. Effects of thyroid hormones on behavior of yearling Atlantic salmon (*Salmo salar*). J. Fish. Res. Board Can. 31: 1787–1790.

La nage, le comportement agressif et l'orientation à contre-courant de saumons atlantiques (*Salmo salar*) de l'année, injectés de 6.43×10^{-11} M de thyroxine, sont nettement plus faibles que ceux de témoins traités au solvant seul. Deux concentrations de triiodothyronine (7.43×10^{-11} M; 7.43×10^{-10} M) produisent des effets semblables mais moins prononcés.

Etant donné que la migration des smolts est accompagnée de modifications semblables du comportement, nous émettons l'hypothèse que les hormones thyroïdiennes peuvent jouer un rôle dans le déclenchement des tendances migratoires chez le saumon atlantique.

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IN freshwater streams, Atlantic salmon (*Salmo salar*) establish and maintain bottom-feeding territories (Kalleberg 1958) by displaying aggressive behavior (Keenleyside and Yamamoto 1962; Symons 1968, 1970). At the end of their stream residency, salmon undergo smoltification and migrate to the sea. Although the cause of behavioral changes transforming an aggressive, stream-dwelling fish to a schooling, migrating one is not known, several investigators have suggested a possible role of the thyroid gland in the physiology associated with smoltification. Hoar (1939), Fontaine et al. (1952), and Leloup and Fontaine (1960) observed that the thyroid gland of the Atlantic salmon becomes hyperactive during the smoltification period. Eales (1965) showed that yearling steelhead trout (*S. gairdneri*), which do not normally smoltify, have a less pronounced spring increase in thyroid activity than 2-yr-old potential smolts. Silvering of the flanks, which characterizes the smolt, has been induced prematurely in steelhead trout (Robertson 1949), Atlantic salmon, and brown trout (*S. trutta*) (Landgrebe 1941), with injections of mammalian thyroid extracts. Baggerman (1963) demonstrated an increased salinity preference in TSH-treated juvenile Pacific salmon (*Oncorhynchus kisutch* and *O. gorbuscha*).

In the absence of data relating thyroid hormones to behavior of young Atlantic salmon, we investigated the effects of two such hormones (thyroxine and triiodothyronine) on swimming activity, aggressive behavior and rheotactic behavior, each of which undergoes a change at the time of smoltification and migration.

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Materials and methods — Yearling Atlantic salmon were obtained from the Antigonish Fish Culture Station, Frasers Mills, N. S. They were held in 400-liter fiberglass "living stream" tanks (Frigid Units, Inc., Toledo, Ohio) at 12 ± 1 C on a 16L:8D photoperiod and fed Purina Trout Chow twice daily (0815 and 1515). The water current was approximately 12 cm/s at the surface, and the water level was maintained at a depth of 25–28 cm.

The fish (mean length 15.4 cm) were injected intraperitoneally (without anesthesia) with either hormone or solvent as follows: L-thyroxine, sodium salt or L-3, 3′-5-triiodothyronine, sodium salt (T_3) was dissolved in a solvent consisting of 2 vol EtOH: 1 vol 0.5N NaOH and then diluted 1:20 with 0.6% NaCl. Final concentrations of 6.43×10^{-11} M L-thyroxine, 7.43×10^{-11} M, or 7.43×10^{-10} M T_3 was injected in a volume of 0.1 ml. Control fish were injected with 0.1 ml of the solvent. Additional injections were given 2, 6, and 10 days later.

On day 6, six control fish and six treated fish were transferred to separate observation tanks. The observation tanks were similar to the holding tanks except that the former had a gravel substrate and were 100 cm × 57 cm with 25–28 cm of water. The behavior of the fish was observed through a glass window on one side of the stream tanks on days 7, 8, 9, 11, 12, and 13, in the experiments with T_3 and on days 7, 8, 9, and 11, in the thyroxine experiment. The observer was obscured behind a screen of black cloth in a darkened room.

All observations were made in July and August, at which time the thyroid gland of juvenile Atlantic salmon (held under simulated summer conditions) is expected to be relatively inactive (Hoar 1939). Control fish and treated fish were observed during two daily 25-min periods each in the early morning and late afternoon, at least 40 min after feeding, because some components of aggressive behavior are enhanced immediately after feeding (Keenleyside and Yamamoto 1962).

TABLE 1. Effects of thyroid hormones on swimming activity and aggressive behavior of Atlantic salmon (*Salmo salar*). Numbers in swimming activity column refer to number of grid lines crossed by fish in tank during two daily 10-min periods. Aggressive behavior column refers to number of aggressive acts observed in two daily 15-min periods. Six fish were used per test. Thyroxine data was averaged over 4 days and T_3 data over 6 days.

Treatment	Swimming activity Mean ± SD	Aggressive behavior Mean ± SD
Thyroxine (6.43×10^{-11} M)	26.0±25.6[a]	5.5±3.4[b]
Control (solvent only)	110.0±46.0	27.8±8.9
Low T_3 (7.43×10^{-11} M)	62.2±57.6	12.7± 8.8[a]
Control (solvent only)	109.8±52.2	33.2±14.7
High T_3 (7.43×10^{-10} M)	97.2±74.3[b]	60.5±89.6
Control (solvent only)	304.8±44.5	41.8±32.4

[a]Probability of greater *t* value 0.05 (two-tailed).
[b]Probability of greater *t* value 0.01 (two-tailed).

TABLE 2. Orientation of fish with respect to water current. Direct upstream orientation is represented by 0 degrees. Values indicate total number of fish falling in each orientation class before and after two daily 25-min observation periods. Thyroxine data was summed over 4 days and T_3 data over 6 days.

Treatment	Orientation classes 0±45°	90±45°	180±45°	Probability (Chi-square)
Thyroxine (6.43×10^{-11} M)	52	28	16	≤0.05
Control (solvent only)	94	2	0	
Low T_3 (7.43×10^{-11} M)	130	14	0	>0.05
Control (solvent only)	125	13	6	
High T_3 (7.43×10^{-10} M)	67	41	36	≤0.05
Control (solvent only)	112	32	0	

The bottom of each tank was divided into 12 equal areas (0.025 m^2 each). Swimming activity was measured by cumulating the number of grid lines crossed by fish during the first 10 min of each observation period. Means were calculated for each group by averaging over the total number of observation days. During the remaining 15 min of the observation period, aggressive behavior patterns including charging, nipping, chasing, approaching, frontal display, and lateral display (Keenleyside and Yamamoto 1962; Symons 1968) were recorded verbally on tape. All aggressive acts were totalled daily for each group of fish. The mean of these over the total number of observation days gave an index of aggressive behavior.

Just prior to and after the 25-min observation periods the orientation of each fish relative to the current was recorded. The directions were classified as being upstream ($0 \pm 45°$), perpendicular ($90 \pm 45°$), or downstream ($180 \pm 45°$). These were then summed for each class over the days of observation in each group.

Results — In general, there was a decrease in the swimming activity of hormone-treated fish compared with that of control fish (Table 1). This reduction is significant ($P \leq 0.05$) for thyroxine-treated fish and highly significant ($P \leq 0.01$) for fish treated with the higher concentration of T_3. Highly significant ($P \leq 0.01$) and significant ($P \leq 0.05$) reductions in aggressive behavior were demonstrated by fish treated with thyroxine and the lower concentration of T_3, respectively, compared with the controls. Fish treated with the higher concentration of T_3 demonstrated a nonsignificant increase in aggressive behavior. Upstream orientation of fish treated with thyroxine and T_3 at the higher concentration was significantly less ($P \leq 0.05$) than that of controls (Table 2); the lower concentration of T_3 had no significant effect.

Discussion — The results of this study indicate that thyroid hormones have an effect on various aspects of the behavior of yearling Atlantic salmon in an artificial stream. In all experiments, the level of swimming activity of the treated fish was reduced compared to that of the control fish. In contrast to these findings, an increase in the rate and/or amount of swimming activity has been reported in *Oncorhynchus keta* (Hoar et al. 1952), *O. kisutch*, *O. nerka*, *Carassius auratus* (Hoar et al. 1955), *Poecilia reticulata* (Sage 1968), and *Gadus morhua* (Woodhead 1970) after treatment with thyroxine. Perhaps yearling Atlantic salmon react differently to thyroid hormones. Although the possibility that some of the reported findings may be pharmacological effects cannot be conclusively ruled out, the hormone dosages we used fell within the range of physiological levels reported for brook trout by Eales (1974).

In preparation for its downstream migration, the Atlantic salmon must abandon its bottom-feeding territory. A reduction in swimming activity that would lead to a decrease in social encounters, may accomplish this. A direct reduction in aggressive behavior could also accomplish this, and in addition, would permit an increase in migratory schooling. Hoar (1951) observed just such a reduction in the aggressive behavior of territorial coho salmon (*O. kisutch*) during smoltification, and our results show that in Atlantic salmon thyroid hormones could be the cause of this reduction.

Atlantic salmon parr, in a nonmigratory stage, orient against the current in artificial streams (Kalleberg 1958) and in rivers (Keenleyside 1962). Migrating smolts swim with the water current in circular tanks (Kalleberg 1958) and in rivers (Stasko et al. 1973). Upstream orientation of our fish treated with 6.43×10^{-11} M thyroxine and 7.43×10^{-10} M T_3 was significantly reduced.

Minimal requirements for downstream migration of Atlantic salmon would appear to include a reduction in aggressive behavior, and a downstream orientation. From the present data, it is suggested that thyroid hormones may be involved in the behavioral changes associated with migration in Atlantic salmon. It remains to test how these findings in an artificial stream, relate to the migratory behavior of salmon in natural streams.

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Occurrence of Hematozoa in Atlantic Salmon (*Salmo salar*) Smolts and Adults in an English River

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MCCARTHY, D. H. 1974. Occurrence of hematozoa in Atlantic salmon (*Salmo salar*) smolts and adults in an English river. J. Fish. Res. Board Can. 31: 1790–1792.

The percentages of hemoflagellates found in single samples of Atlantic salmon (*Salmo salar*) from the River Exe, Devon, in 1971 (122 fish) and 1973 (50 fish) were 33 and 32; of hemogregarines, 18 and 22. The percentages for 21 adults taken in the estuary in August 1971 were 14 and 0, respectively; in 9 adults taken in the estuary in March 1972 and in 9 taken in fresh water in November 1971, no hematozoa were found. Statistical analysis of hemoglobin data showed no significant difference between parasitized and healthy fish. Neither the specific identities nor the vectors of the hematozoa were determined.

MCCARTHY, D. H. 1974. Occurrence of hematozoa in Atlantic salmon (*Salmo salar*) smolts and adults in an English river. J. Fish. Res. Board Can. 31: 1790–1792.

Les pourcentages d'hémoflagellés trouvés dans des échantillons uniques de saumons atlantiques (*Salmo salar*) capturés dans la rivière Exe, Devon, en 1971 (122 poissons) et 1973 (50 poissons) sont de 33 et 32; ceux d'hémogrégarines, 18 et 22. Les pourcentages, chez 21 adultes capturés dans l'estuaire en août 1971, sont de 14 et 0 respectivement; dans 9 adultes capturés dans l'estuaire en mars 1972 et 9 pris en eau douce en novembre 1971, aucun hématozoaire n'a été trouvé. L'analyse statistique des données sur l'hémoglobine ne démontre pas de différence significative entre les poissons infestés et les poissons sains. L'identité spécifique, non plus que les vecteurs des hématozoaires n'ont pu être déterminés.

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ALTHOUGH both hemoflagellates and hemogregarines have been reported from Pacific salmon (*Oncorhynchus* spp.) and other salmonids (Becker and Katz 1965a; Katz 1949, 1951; Katz et al. 1966; Wales and Wolf 1955), they have only recently been discovered in Atlantic salmon (*Salmo salar*) smolts (Conroy and McCarthy 1972).

This report gives results of further sampling to assess the incidence of hematozoa in both adults and smolts of the Atlantic salmon from the River Exe, Devon. In addition, hemoglobin values for both parasitized and healthy fish were compared statistically in an attempt to detect a pathological effect which might be attributable to the presence of hematozoa. Five samples were studied: 122 and 50 migrating smolts taken in May of 1971 and 1973, respectively; 21 and 9 returning adults taken in the estuary in August 1971 and March 1972, respectively; and 9 returning adults taken in fresh water in November 1971.

Methods — All fish were caught by netting. Smolts were held in tanks for up to 24 h before bleeding. With the exceptions that small fish were bled from a severed caudal fin and larger fish were not anesthetized, blood collection procedures and hematological techniques were as described previously (McCarthy et al 1973).

Results and discussion — The percentages of smolts in the 1971 and 1973 samples infected with hematozoa of both groups (Table 1) were remarkably similar. This observation, together with a relatively high percentage of dual infections by the same parasites in each sample, suggests that the parasites may have the same, or a similar, vector. Hemoflagellates, but not hemogregarines, were found in the 1971 sample of returning adults taken in the estuary. Although this is the first report of hemoflagellates in adult Atlantic salmon, they have already been found in adult Pacific salmon (Katz et al 1966). If rhynchobdellid leech, or other, vectors for hemoflagellates are absent from estuarine waters then the presence of these parasites in adults suggests that they can withstand their host's physiological change to a marine environment.

TABLE 1. Percentages of Atlantic salmon (*Salmo salar*) infected with hematozoa and saprolegniasis, along with mean hemoglobin (*g*/100 *ml*) and hematocrit (%) values for adults and hemoglobin values for smolts.

Sample	Hemoflagellates	Hemogregarines	Both parasites	Uninfected	*Saprolegnia* sp.
			Smolts		
1971, May (122 fish)					
Percentage infected	33	18	11	38	64
Hemoglobin	9.0±1.98[a]	9.4±2.07	9.1±2.25	9.2±2.17	8.0±2.20
1973, May (50)					
Percentage infected	32	22	8	38	58
Hemoglobin	9.9±1.90	10.2±2.01	10.2±1.99	9.8±2.07	8.4±1.97
			Returning adults, in estuary		
1971, August (21)					
Percentage infected	14	–	–	86	–
Hemoglobin	10.6±1.71	–	–	10.8±1.42	–
Hematocrit	63.7±6.81	–	–	64.9±7.05	–
1972, March (9)					
Percentage infected	–	–	–	100	–
Hemoglobin	–	–	–	5.7±0.44	–
Hematocrit	–	–	–	45.8±4.3	–
			Returning adults, in fresh water		
1971, November (9)					
Percentage infected	–	–	–	100	100
Hemoglobin	–	–	–	3.8±0.90	–
Hematocrit	–	–	–	30.2±13.0	–

[a]Standard deviation.

The absence of hematozoa in the returning adults taken in fresh water (Table 1) is noteworthy in view of the positive results for estuarine fish taken prior to entering a river known to contain infected fish and hematozoa vectors. Although examination of nine leeches (*Pisicola* spp.) taken from these fish and known (Becker and Katz 1965b) to be vectors of *Trypanoplasma* (= *Cryptobia*) *salmositica* yielded no hematozoa, failure to detect hematozoa in the fish may have been due to the small number examined.

The numbers of hemoflagellates found in blood samples from individual fish varied widely; infections in smolts, for example, ranged from heavy (1–2/100 erythrocytes) to light (.01/100). The numbers of hemogregarines on the other hand were invariably light (.001–.01/100).

Dr C. D. Becker (Battelle Pacific Northwest Laboratories, Washington, personal communication), although unable to positively identify the species of either group of hematozoa, noted that the hemogregarines were similar in size and morphology to *Haemogregarina irkalukpiki* Laird 1961. Concerning the hemoflagellates, Becker considers that all cryptobiids found in western Europe may be encompassed by a single species, *Trypanoplasma* (= *Cryptobia*) *borreli* (Laveran and Mesnil 1901).

Statistical analysis of hemoglobin data from smolts and adults (Table 1) showed no significant difference ($P > 0.05$) between parasitized and healthy fish. However, infection with saprolegniasis, which occurs annually among the Exe smolt "run" causing high mortalities, was associated with a hemoglobin value that was reduced significantly. It is of interest, therefore, that statistical analysis failed to reveal a significant correlation between the incidences of either group of hematozoa, or both, and saprolegniasis. These results indicate that natural infections of migrating Atlantic salmon smolts with hematozoa did not handicap the fish to a significant degree. Moreover, in the study, observation of refractive granules in the cytoplasm of stained hemoflagellates, confirmed by Becker (personal communication), suggested a host-induced reaction (Becker and Katz 1969). The occurrence of this reaction, together with an apparent lack of pathogenicity, would suggest that the Exe smolt was an "unnatural" host to the hemoflagellates. It must be remembered, however, that *Trypanoplasma salmositica* is extremely pathogenic, by artificial infection, to one of its natural hosts, the coho salmon (*O. kisutch*) (Putz 1972). In addition, although coho salmon appear to tolerate natural infections with hemoflagellates, chinook salmon (*O. tshawytscha*) populations may be decimated by "natural infections" (M. Katz personal communication). Although a large difference in hematological values was found between estuarine fish sampled in August 1971 and March 1972 (Table 1), this could not be attributed to the presence of hematozoa. The marked reduction in hematological values of adult freshwater fish, compared with those for estuarine fish, was most likely due to infection with saprolegniasis, although progressive maturation of gonads is known to reduce certain blood values in migrating salmon.

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Circulation in Trout Gills: Presence of "Blebs" in Afferent Filamental Vessels

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FROMM, P. O. 1974. Circulation in trout gills: presence of "blebs" in afferent filamental vessels. J. Fish. Res. Board Can. 31: 1793–1796.

Casts of the gill vasculature of rainbow trout (*Salmo gairdneri*) perfused with Microfil were examined visually. The most conspicuous feature seen was enlargements, called blebs, of the afferent filamental vessels that were located in the outermost region of the interfilamental septa. Several lamellae were supplied by profusely branched afferent lamellar vessels, whereas most lamellae were individually connected to the efferent filamental vessel. Vessels contained in the septal area and those that ran parallel to the long axis of the filaments are also described and possible functions of the blebs are discussed.

FROMM, P. O. 1974. Circulation in trout gills: presence of "blebs" in afferent filamental vessels. J. Fish. Res. Board Can. 31: 1793–1796.

Des moules de vaisseaux branchiaux de truite arc-en-ciel, (*Salmo gairdneri*) obtenus après perfusion de Microfil, ont été soumis à un examen visuel. Des élargissements, appelés phlyctènes, des vaisseaux filamentaux afférents situés dans la région la plus externe des septa interfilamentaux sont le caractère le plus saillant observé. Plusieurs lamelles sont desservies par des vaisseaux lamellaires afférents abondamment ramifiés, alors que la plupart des lamelles sont réunies individuellement au vaisseau filamental efférent. Nous décrivons également les vaisseaux contenus dans la région septale et ceux dont le parcours est parallèle au grand axe des filaments, et nous examinons la fonction possible des phlyctènes.

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A variety of techniques have been used to study circulation in fish gills and much of the information has been summarized recently (Hughes and Morgan 1973). Blood from the ventricle is delivered via the ventral aorta to the gill filaments by way of the afferent gill and afferent filamental blood vessels. There appear to be three possible routes by which afferent filamental blood can reach efferent filamental vessels: 1) via channels in secondary lamellae (respiratory pathway), 2) directly from the afferent to the efferent filamental vessel at the tip of the filament, and 3) through a central filament space (nonrespiratory pathway). Evidence for the latter was presented by Steen and Kruysse (1964) and by Richards and Fromm (1969), but more recently its presence has been questioned (Hughes and Morgan 1973; Morgan and Tovell 1973; Gannon et al. 1973; Cameron 1973; Vogel et al. 1973). Hughes and Morgan (1973) suggest that the presence of a nonrespiratory shunt is not required to explain the present physiological findings; however, the argument for the presence of this shunt in the gill (Steen 1963) still seems valid. He suggested that it might be difficult for euphysoclistous fishes to utilize the oxygen reabsorbed from the swim bladder with an absence of nonrespiratory shunts in the gill. Reports of low values of 50–75% saturation of hemoglobin in arterial blood (Steen and Kruysse 1964; Eddy 1974) offer some possible support for the presence of nonrespiratory shunts in teleost gills.

The work reported is an outgrowth of studies on circulation in trout gills injected with latex and examined after acid digestion (Richards and Fromm 1969). Later Microfil, a vulcanizing liquid silicone compound that flows through vessels of capillary size much more readily than latex, became available. This report deals with observations on gill circulation in specimens infused with this compound.

Materials and methods — Rainbow trout (*Salmo gairdneri*) were anesthetized with MS-222 and placed ventral side up in a V-shaped trough, and an incision was made to expose the heart and bulbus arteriosus. Freshly mixed Microfil (2.5 ml MV 112 white, 2.0

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ml MV diluent, and 0.135 ml curing agent) (Canton Bio-Medical Products, Inc., Boulder, Colo.) was drawn into a 5-ml syringe and infused via a polyethylene cannula (PE 90) that had been inserted and secured into the ventral aorta. Gentle pressure on the syringe plunger was applied manually and excessive distention of the ventral aorta avoided by extending the infusion over a period of 12–15 min. The infusion pressure was not recorded. About 5 ml Microfil was injected into fish weighing around 200 g, a much larger volume than that required to fill the gill vasculature. This large volume was infused in an attempt to get Microfil through the pseudobranch and into the eye of trout for another series of experiments. Significant amounts passed through the gills and appeared in vessels of the musculature and the splanchnic area. After injection, the specimens were stored in a refrigerator overnight to allow complete polymerization of the Microfil. They were then cleared in methyl salicylate (Merck), and selected casts with adhering cleared tissue were submerged in methyl salicylate and photographed.

Observations — The trout circulatory system from the ventricle to the secondary lamellae fit the classical description except that the efferent gill vessel was found to leave the arch a short distance below the upper limit of the gill, leaving a cul-de-sac extension to drain blood from vessels of the more dorsally located filaments. In some cases a pair of afferent filamental vessels shared a common origin from the afferent gill vessel. There appeared to be no significant shunting of the silicone material from the afferent to the efferent filamental vessels via a central filament space. It is clearly shown in Fig. 1 that there is rather profuse branching of the afferent lamellar vessels with as many as five secondary lamellae being connected to a single vessel. The efferent lamellar vessels were of somewhat smaller diameter and much shorter than the afferent lamellar vessels and usually a single lamella was drained by one vessel. Occasionally two, but never more than two, efferent lamellar vessels united prior to joining the efferent filamental vessel.

Many vessels that arise from the base of the afferent filamental or from the afferent gill vessel were observed in the interfilamental septal area. Vessels on either side of the septum (Fig. 1) can be seen to loop toward the base of the filament and then leave the septal area and run parallel to the afferent filamental vessel. Although it is not as clearly visible in Fig. 1, a second somewhat larger vessel, distal to the bleb, also ran parallel to the afferent filamental vessel toward the tip of the filament. It appeared as if this vessel might provide cross connections between afferent lamellar vessels at the point where they empty into the space surrounding the pillar cells. Another septal vessel of smaller diameter than the efferent filamental vessels was found in several but not all gill arches. This vessel was located in the basal portion of the septal area and ran parallel to the afferent gill blood vessel. Several additional short intraseptal vascular connections between successive pairs of filaments on a gill arch were also noted.

Perhaps the most conspicuous feature of the gill casts was the presence of blebs. These aneurysm-like enlargements of the afferent filamental vessels were located in the most distal part of the septal area (Fig. 1 and 2), roughly one-third of the distance from the base to the tips of filaments. The casts appeared as if those on apposing filaments would make contact with one another when fully distended but no vascular connections between blebs were found.

It appears presumptive that the blood vessels in the interfilamental septal area and those that run parallel to the afferent filamental vessels are not involved in gas exchange. The function of the filamental blebs, on the other hand, is even more speculative.

Discussion — The circulatory system of fish can be depicted as a "double windkessel model" (Satchell 1971) with dampening of pulsatile flow through the gills being provided by the distensibility of the ventral aorta and afferent gill vessels. It would appear that the blebs present along the afferent filamental vessels in trout gills would augment this effect.

In the casts observed there appeared to be no flow of Microfil into a central filamental bypass of the secondary lamellae. It is reasonable to assume that injected fish were ratherly severely stressed; hence, their plasma catecholamine level should have been very high. Epinephrine is known to cause a high blood flow through lamellae (Steen and Kruysse 1964; Richards and Fromm 1969); thus, under the circumstances of injection one would not expect to get any appreciable flow of blood into a filamental shunt. Similar conditions, i.e. use of stressed fish, may likewise have prevented others (Hughes and Morgan 1973; Morgan and Tovell 1973; Gannon et al. 1973; Cameron 1973) from observing blood flow in a nonrespiratory bypass of the secondary lamellae.

The recruitment of additional secondary lamellae to act as respiratory units has been discussed by Morgan and Tovell (1973) who suggest that this action could be passive, resulting from increased pressure on the afferent side of the filament, or active, by relaxation of vascular smooth muscle. Davis (1972) states that infusion of adrenalin caused more blood to flow into lamellae, especially those located toward the gill filament tips. Just how

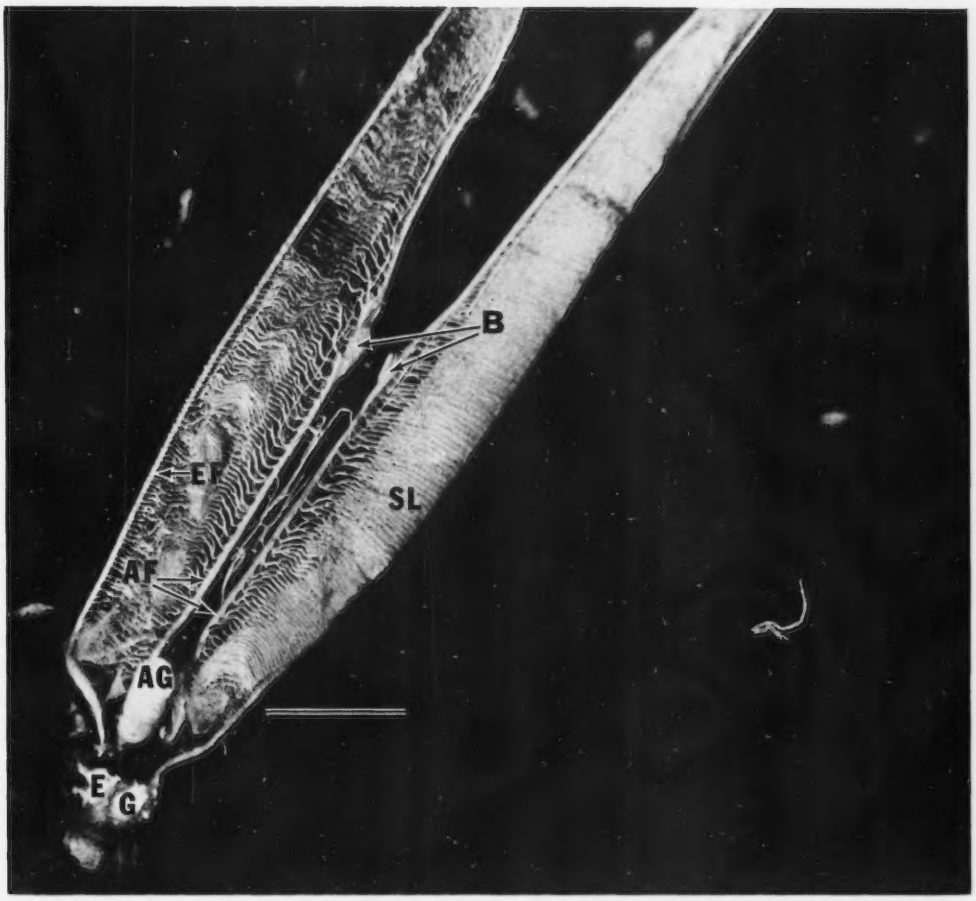

FIG. 1. Microfil cast of a single pair of gill filaments from rainbow trout (*Salmo gairdneri*). AG, afferent gill vessel; AF, afferent filamental vessel; EG, efferent gill vessels; EF, efferent filamental vessel; B, afferent filamental blebs; SL, secondary lamellae. Calibration bar = 1 mm.

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FIG. 2. Microfil casts of four rainbow trout filaments from one hemibranch showing afferent filamental blebs located in the outermost edge of the interfilamental septal area. Calibration bar = 1 mm.

the blebs might participate in recruitment is not known. It should be emphasized again that no vascular connections were found between filamental blebs on the same or opposite hemibranch which could serve as a bypass for the distally located secondary lamellae. I am not aware of any anatomical specialization in the afferent filamental vessels that would alter the perfusion of secondary lamellae distal to the blebs, and we have made no attempt to identify possible nerve connections to or sensory cells on the blebs. If constriction of the afferent filamental vessels immediately distal to the blebs occurs when the demand for oxygen by the animal is minimal, one could visualize the blebs as being responsible for providing a smooth, continuous flow of blood through the operational secondary lamellae located on the basal portion of filaments. It is beyond the scope of this paper to discuss the pros and cons of smooth vs. pulsatile blood flow with respect to respiratory exchange but it does seem appropriate to suggest that the blebs observed in gills of rainbow trout must play an important role in dampening the pulsatile outflow of blood from the heart.

With suitably placed flow probes Jones et al. (1974) have shown that "dorsal" and "ventral aortic" flow was similar in a hydraulic model of circulation in fish, whereas flow in the intact cod was different, which indicated a considerable compliance (between the two flow probes) in excess of that operating in the model. Perhaps structures similar to trout blebs are also present in cod and provide an additional compliance in the gills. The presence of blebs in *S. gairdneri* may at least partially explain why the gill blood volume of 3.8 ml/kg body weight as determined by Stevens (1968) is considerably higher than that of *S. trutta* and the cod as calculated by Jones et al. (1974) from Hughes' (1966) data.

In these experiments we were aware that perfusion pressure during casting was rather critical, especially with respect to flow into the central filament space between afferent and efferent filamental vessels. It was not practical to record perfusion pressure but care was taken to avoid excessive distension of the ventral aorta during infusion. There was no evidence of the application of excessive pressure, e.g. rupture of gill vessels; thus, we believe that the casts represent fairly accurately the size and shape of vessels and blebs in trout gills.

Using a dissecting microscope and intense direct illumination we have observed a turbulent, pulsatile flow of blood into the bleb area of rainbow trout gills in situ and the frequency of this flow into the blebs was found to correlate perfectly with a simultaneous recording (ECG) of heart rate. Also when a large nerve carrying fibers to an isolated-perfused gill arch was stimulated, adduction of the filaments occurred, accompanied by an increase of about 15% in the perfusion pressure. When stimulation was extended for as long as 60 s the increased pressure rapidly subsided, lasting for only 15–20 s. It is tempting to speculate that contraction of the adductor muscles may have increased tissue pressure surrounding the blebs and facilitated their emptying. If rhythmical isotonic or isometric contraction of these muscles occurred synchronously with heart beat, the pumping effect would be analagous to that of gill hearts that aid in forcing blood through the gill vasculature. Unfortunate for this idea is the observation by Bijtel (1949) that adduction movement of the filaments (which might not occur with isometric contraction of adductor muscles) appears sporadically and then only in connection with coughing movements.

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Composition of Ovarian Fluid and Eggs of the University of Washington Strain of Rainbow Trout (*Salmo gairdneri*)[1]

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SATIA, B. P., L. R. DONALDSON, L. S. SMITH, AND J. N. NIGHTINGALE. 1974. Composition of ovarian fluid and eggs of the University of Washington strain of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 31: 1796–1799.

The concentrations of major constituents of ovarian fluid were found to be lower than reported values for the same constituents in blood and urine of rainbow trout (*Salmo gairdneri*). Glucose and total bilirubin were significantly correlated ($P < 0.05$) to hatchability, whereas LDH was negatively correlated ($P < 0.05$).

Rainbow trout egg proteins are highly nutritional and contain a higher proportion of essential amino acids than do eggs or human or cows' milk.

SATIA, B. P., L. R. DONALDSON, L. S. SMITH, AND J. N. NIGHTINGALE. 1974. Composition of ovarian fluid and eggs of the University of Washington strain of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 31: 1796–1799.

On constate que la concentration des constituants majeurs du fluide ovarien est plus faible que les valeurs signalées pour les mêmes constituants du sang et de l'urine de la truite arc-en-ciel (*Salmo gairdneri*). Le glucose et la bilirubine totale ont une corrélation significative ($P < 0.05$) avec le succès de l'éclosion; par contre, la LDH a une corrélation négative ($P < 0.05$).

Les protéines d'oeufs de truite arc-en-ciel ont une haute valeur nutritive et contiennent une plus forte proportion d'acides aminés essentiels que les oeufs, le lait humain ou le lait de vache.

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LITTLE has been reported on the composition of fishes' ovarian fluid and specifically that of rainbow trout (*Salmo gairdneri*). Hayes et al. (1946) measured the concentrations of Na, P, K, Ca, Mg, and Cl in coelomic fluid of Atlantic salmon (*S. salar*) and followed the concentrations of these ions in developing yolks and embryos. They observed that the levels of these ions remained relatively constant throughout embryonic development. At hatching, however, there was a diminution of K and an increased concentration of Ca and P. No attempts have been made to study the relationship of rainbow trout ovarian constituents to fertility or hatchability of the ova. Despite increased use of salmonid eggs for human nutrition in parts of North America, Asia, and Europe, only limited

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information is available concerning the gross composition and amino acid content of rainbow trout eggs. However, the amino acid content of Pacific salmon eggs has been followed through different stages of maturity (Seagran et al. 1954).

The purposes of this study were to determine the chemical composition of rainbow trout ovarian fluid and whether or not a relationship existed between ovarian fluid composition and egg hatchability, to obtain data on the gross composition and amino acid content of ova produced by the University of Washington strain of rainbow trout, and to compare the quality of rainbow trout egg protein with other protein supplements used in human foods.

Materials and methods — Two-year-old University of Washington strain rainbow trout (55.70 ± 2.45 cm, 3.32 ± 0.50 kg, at the time of spawning) were used in this study (Donaldson and Olson 1957; Donaldson 1971). They were fed on Oregon Moist Pellet type diet (modified University of Washington diet) at the rate of 2% of their body weight on alternate days for 9 mo prior to use in this study. Mean water temperature during the growth period was 13.5 + 6.30 C (range 7–25 C).

Ovarian fluid — When the fish became sexually mature ovarian fluid was collected by applying pressure manually to the abdomen of the fish and allowing the fluid and any ova to drip directly into sterilized 30-ml vials. Ova (if any) were removed and the sample was sealed, packed in ice, and immediately transported to a local commercial laboratory (Scientia Laboratories, Seattle, Wash.), where the calcium, cholesterol, inorganic phosphates, total bilirubin, albumen, total protein, uric acid, urea nitrogen, glucose, lactic dehydrogenase, and alkaline phosphatase of each specimen were determined, using a Technicon SMA-1260 autoanalyzer. The concentrations of Cl, Mg, Na, and K in the fluid were determined by atomic absorption spectrophotometry and flame photometry.

Egg taking and incubation of eggs — After the ovarian fluid was collected, each fish was carefully strip-spawned. The ova were fertilized without water (one female × one male per lot, a total of 24 lots) and placed randomly in separate trays of a Heath incubator. This unit of 24 trays was surrounded by two rows each of trays at the top and at the bottom and a column of trays on each side. The surrounding trays contained no ova. The flow rate of water was 18 liters/min. During incubation (9.5 ± 1.3 C) the ova were shielded from direct light by black plastic covers and disinfected three times (for control of fungus) by a flush treatment of malachite green at a concentration of 1:500,000 for 1 h (Wood 1968). When the ova became water-hardened, 300 were taken from each lot. The ova were dried with blotting paper and the weight of each of three 100-egg samples determined. The total number of ova in each lot was determined by the Von Bayer method (Von Bayer 1908). From data on the total number of ova in each lot, the average sizes (diameter millimeters) of the ova were calculated.

Incubating ova were examined daily throughout development. Dead ova were removed and the numbers recorded until approximately the time of yolk-sac absorption and the beginning of feeding. The percentage survival during the uneyed egg stage, during the eyed egg stage and during the alevin stage, as well as the total survival from fertilization to the time of yolk-sac absorption, were calculated.

Gross composition, caloric content, and amino acid content of ova — Ova from two females were pooled and ground to a fine homogenate. Weighed fractions were used in triplicate to determine water, protein, ash, and lipid content by Standard Methods (AOAC 1965). Values for total survival and gross composition of ova were transformed, using arcsine $\sqrt{\text{percentage}}$. Computer program BMDO3R (Dixon 1970) was used to determine correlations between these variables. Analysis of amino acid content of ova were carried out at the University of Washington, Department of Biochemistry. The analyses were done by automated cation-exchange chromatography (Benson and Patterson 1971; Moore and Stein 1963).

Results and discussion — Ovarian fluid — The fluid collected was clear, limpid, and slightly translucent. There was a wide range of some constituents within individual samples, the cause of which is not

TABLE 1. Chemical composition of rainbow trout (*Salmo gairdneri*) ovarian fluid and correlation between total embryonic survival and the components of ovarian fluid.

Component	Units	Amount	*r* values
Ca^{++}	mg %	8.00±1.04[a]	−0.26
Inorganic phosphate		11.49±4.41	−0.34
Glucose		5.25±3.30	0.55[b]
Blood urea nitrogen		0.93±0.68	−0.06
Uric acid		0.18±0.07	0.22
Cholesterol		2.00±0.19	−0.01
Total protein	g %	0.04±0.05	0.18
Albumen	"	0.06±0.02	−0.11
Total bilirubin	mg %	0.05±0.05	0.59[b]
Alkaline phosphatase	IU	7.00±3.89	−0.18
Lactic dehydrogenase	"	5.50±2.05	−0.63[b]
SGOT/340	"	0.00	–
Na	Meq/L	5.50±3.11	−0.13
K	"	1.25±0.19	−0.13
Cl	"	8.00±0.16	0.27
Mg	"	1.48±1.05	−0.02

[a]Mean ± SD.
[b]Significant at 0.05 level of probability.

TABLE 2. Characteristics of rainbow trout eggs and correlation matrix for these characteristics. (N = 24.)

Characters	Quantities	Egg no.	Egg size	Egg wt	Egg hatchability	% protein in egg	% lipid in egg	% ash in egg	% moisture in egg
Egg number	10,019±1,735.73[a]	10000	−.3984[b]	−.4677[b]	−.1895	−.1077	−.0186	.1328	.0931
Egg size (*mm*)	4.46±0.19		10000	.4908[b]	.1199	.2645	.1295	.2559	.2868
Total weight (*mg*)	0.45±0.03			10000	.2976	.2618	.1358	.0029	−.3145
Total embryonic survival	81.05±6.50				10000	.7248[b]	.3628	−.1955	−.6491[b]
% protein in egg	24.11±0.48					10000	−.5161[b]	.0601	−.6903[b]
% lipid in egg	3.84±0.22						10000	.0259	−.3685
% ash in egg	1.33±0.08							10000	.0351
% moisture in egg	66.18±0.46								10000

[a]Mean ± SD.
[b]Significant at 0.05 level of probability.

TABLE 3. Comparisons of amino acid profile to that of other protein supplements and FAO amino acid reference pattern.

	Grams/100 g protein					
Amino acid	Rainbow trout whole eggs[a]	Chinook salmon eggs[b]	Eggs[c]	Human milk[c]	Cows' milk[c]	FAO pattern[c]
Lysine	8.3±.01	8.3	6.6	6.6	7.9	4.2
Methionine	2.1±.02	3.0	2.1	3.1	2.3	2.2
Total sulfur AA	–		5.4	4.3	3.4	4.2
Threonine	6.0±.02	5.8	5.0	4.5	4.7	2.8
Tryptophane	–	0.8	1.7	1.6	1.4	1.4
Valine	8.9±.02	7.1	7.4	6.3	7.0	4.2
Phenylalanine	5.2±.01	4.8	5.8	4.4	4.9	2.8
Leucine	7.6±.03	9.8	8.8	9.1	10.0	4.8
Isoleucine	4.7±.01	7.2	6.6	5.5	6.5	4.2
Tyrosine	–	–	5.0	5.5	5.1	2.8
Cysteine	–	–	2.3	2.0	0.9	–
Aspartic	6.8±.02	–				
Serine	5.5±.03	–				
Glutamic	7.6±.04	–				
Proline	3.8±.02	–				
Glycine	4.6±.01	–				
Alanine	4.9±.01	–				
Histidine	2.8±.01	2.5				
Arginine	7.6±.02	7.5				

[a]SE of mean N = 12.
[b]Seagran et al. 1954.
[c]FAO 1957.

known. However, good agreement was obtained between replicate samples, so that the variations observed are believed to be real. There was a large excess of base over acid in the constituents measured, sodium being the dominant basic ion and chloride the major acidic ion (Table 1). The concentrations of the constituents were much lower than those for same constituents in blood and urine of rainbow trout (Holmes and Donaldson 1969). Analyses of the data revealed only a few significant correlations between constituents of ovarian fluid and total percent survival of the ova. Glucose and total bilirubin were significantly correlated ($P < 0.05$) with total ova survival. The enzyme LDH was negatively correlated ($P < 0.05$) with total ova survival (Table 1).

Gross composition and amino acid content of ova — Ova weight was not significantly correlated ($P > 0.05$) with stored up nutrients in the ova (Table 2). A significant negative correlation ($P < 0.05$) existed between protein content and either percent lipid or moisture in the ova. The significant positive correlation ($P < 0.05$) between percent protein in ova and total embryonic survival suggests that proteins are important to embryonic development (Table 2). The position of the trays in the incubator unit had no significant effect ($P > 0.05$) on total embryonic survival.

From the standpoint of protein quality, as determined by essential amino acid content, rainbow trout ova have a nutritive quality equivalent to that of cows' milk, human milk, and whole chicken eggs. All essential amino acids in rainbow trout ova appear in significant excess over those of the FAO reference pattern for human nutrition. Compared to chinook salmon (*Oncorhynchus tshawytscha*) ova, however, the content of rainbow trout ova differed considerably in certain respects (Table 3). Whether these differences are due to strain, nutrition, or species is not known.

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Age Composition and Growth of Yellowtail Flounder (*Limanda ferruginea*) from the Grand Bank

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PITT, T. K. 1974. Age composition and growth of yellowtail flounder (*Limanda ferruginea*) from the Grand Bank. J. Fish. Res. Board Can. 31: 1800–1802.

The commercial yellowtail flounder (*Limanda ferruginea*) fishery on the Grand Bank is comprised of fish age 4–12 with the main contribution from 6–8-yr-olds. Comparison of Grand Bank yellowtail with those reported for the Scotian Shelf indicates similar growth curves. The New England stocks are significantly larger at comparable age probably because of higher water temperatures.

PITT, T. K. 1974. Age composition and growth of yellowtail flounder (*Limanda ferruginea*) from the Grand Bank. J. Fish. Res. Board Can. 31: 1800–1802.

La pêcherie commerciale de limandes à queue jaune (*Limanda ferruginea*) du Grand banc est constituée de poissons âgés de 4 à 12 ans, ceux de 6 à 8 ans y apportant la contribution la plus importante. Une comparaison de la croissance des limandes à queue jaune du Grand banc avec celle signalée sur le plateau néo-écossais donne des courbes semblables. Les stocks de la Nouvelle-Angleterre sont nettement plus grands à un âge donné, probablement à cause de températures de l'eau plus élevées.

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YELLOWTAIL flounder (*Limanda ferruginea*) have recently become of considerable importance to the Canadian east coast fishery, particularly the fishery based in Newfoundland. Total landings from the Grand Bank (ICNAF Subarea 3) increased from 3 to 30 thousand tons from 1965 to 1973 with about 80% of this landed by Canadian vessels. Pitt (1970) suggested that the rapid increase in abundance of yellowtail was related to an increase in bottom temperatures and a drastic reduction in the size of haddock stocks, which were apparently competitors of yellowtail, at least during part of the year.

Growth curves and age composition data of *L. ferruginea* from the Grand Bank are presented and comparisons made with data for the same species from more southern localities — Scotian Shelf (ICNAF Subarea 4) (Scott 1954; Halliday 1973) and the New England area (ICNAF Subarea 5) (Lux and Nichy 1969).

Materials and methods — The validity of otoliths in determining age of yellowtail was well established by Scott (1954) for specimens taken on the Scotian Shelf; however, very few otoliths from the Grand Bank were as distinct as those illustrated by Scott.

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Otolith samples for age interpretation were obtained from both commercial and research vessel catches. Commercial samples were representative, as far as possible, of landed catches. On research vessels the codend of the otter trawl was lined with ½–1½-inch nylon netting. Random or stratified samples were taken, and in all cases age–length keys were used to adjust the otolith readings to the total measured length frequency in the case of the commercial samples and to the total catch for the research vessel data. Von Bertalanffy growth curves were fitted to the age–length data. January–July data were used both for research and commercial growth curves since most of the sampling was done during this period. Mostly whole otoliths were used; however, because of thickening at the center many required grinding to permit proper interpretation of the first year.

Age composition — For the Grand Bank, commercial catches contained fish from age 4 to 12, with the main contribution from ages 6 to 8 (Fig. 1). In research vessel catches 3-yr-olds were also encountered but no 1- and 2-yr-olds were recorded, although a lined net was used.

Halliday's (1973) data for the Scotian Shelf showed an age distribution very similar to recent Grand Bank landings with very few fish of age 4 and under or over age 10. Earlier data by Scott (1954) from the same area showed no 4-yr-olds in

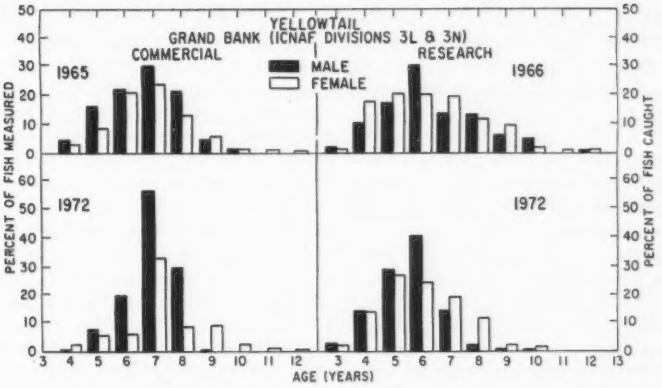

FIG. 1. Age distribution of commercial and research catches of yellowtail flounder (*Limanda ferruginea*) from the Grand Bank (ICNAF Divisions 3L-N).

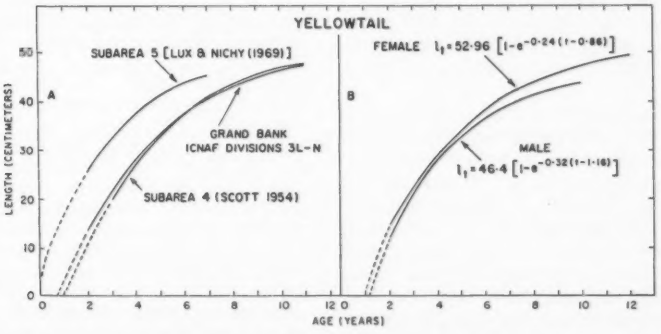

FIG. 2. A, Growth comparison for three northwest Atlantic stocks of yellowtail; B, Growth curves of male and female Grand Bank yellowtail.

TABLE 1. Parameters of von Bertalanffy growth equation for yellowtail flounder (*Limanda ferruginea*) from different localities in the northwest Atlantic.

		Ages fitted (*yr*)	L_∞	K	t_o
Grand Bank commercial	(F)	4–10	52.96	0.24	0.86
Grand Bank commercial	(M)	4–12	46.40	0.32	1.16
Grand Bank commercial	(M+F)	4–12	50.20	0.28	0.63
Grand Bank research	(F)	3–12	48.12	0.29	0.80
Grand Bank research	(M)	3–11	42.07	0.41	1.39
[a]Scotian Shelf research	(M+F)	4–11	52.0	0.26	1.29
[b]New England commercial	(M+F)	2–7	50.0	0.34	–0.26

[a]Calculated from Scott's (1954) data.
[b]Lux and Nichy (1969).

the commercial landings and roughly 85–90% of the landings to be fish of age 7–9 yr. In the New England area, on the other hand, yellowtail are recruited to the fishery at age 2 with commercial landings composed mainly of 3- to 4- and sometimes 5-yr-old fish (Lux 1969).

Growth — Because of slight differences in size at age the sexes were separated (Fig. 2B). However, for comparisons with other areas they were combined (Fig. 2A).

Calculated separate growth curves for males and females (Fig. 2B) indicate slightly different rates of growth between the sexes with females a little larger at comparable age than males, especially beyond age 6.

Curves for males and females combined were compared with those given by Lux and Nichy (1969) for New England areas and also with data presented by Scott (1954) for the Scotian Shelf (Fig. 2A and Table 1). The Grand Bank and Scotian Shelf curves are practically identical but differ from that for the more southerly New England stocks where growth appears to be more rapid (Fig. 2A).

Discussion — The rapid period of growth in the first and second years apparently accounts for the big difference in length at age between Grand Bank (ICNAF Subarea 3) yellowtail and those from the New England area (Subarea 5). Lux and Nichy (1969) showed that yellowtail off New England at the completion of the first full year (April–June) averaged 11.6 cm and in their second year (July–September) averaged 18.5 cm, with a slightly smaller average size for Georges Bank (New England area) fish (18.1 cm). Grand Bank and Scotian Shelf yellowtail do not reach this size until their third year.

Yellowtail on the Grand Bank are near their northern limits and were most abundant at temperatures of 3–4 C (Pitt 1970), although some were caught at –1.0 C and up to 6.5 C. The hydrography of the Scotian Shelf (Hachey 1942; McCracken 1965) suggests that temperatures in depths inhabited by yellowtail are fairly similar to those of the Grand Bank and substantially below those off New England, 4.9 to 12.3 C (Royce et al. 1959).

Gunter (1950), among others, discussed the fact that fish inhabiting high water temperature regions exhibit rapid growth, earlier sexual maturity, and smaller final size than the same species in lower temperature regimes. Yellowtail on the Grand Bank and Scotian Shelf mature at ages 5–6, whereas in the New England area sexual maturity is attained at age 2–3. Royce et al. (1959) and Lux and Nichy (1969) indicated very few fish over age 7 off New England as compared to yellowtail up to age 12 on the Grand Bank, although heavy commercial exploitation could, in part at least, be responsible for this.

Other factors such as population density and interspecific competition could cause differences in growth between regions; however, it is difficult to see how these might apply here.

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A Comparison of Lipids and Fatty Acids of the Ocean Quahaug, *Arctica islandica*, from Nova Scotia and New Brunswick

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Ackman, R. G., S. Epstein, and M. Kelleher. 1974. A comparison of lipids and fatty acids of the ocean quahaug, *Arctica islandica*, from Nova Scotia and New Brunswick. J. Fish. Res. Board Can. 31: 1803–1811.

Lots of ocean quahaugs, *Arctica islandica*, respectively from Nova Scotia and New Brunswick, were compared to see if fatty acid compositions might reflect local food sources and thus explain alleged flavor problems. Lipid contents increased from May to July, but detailed fatty acid comparisons including those from flesh and hepatopancreas, gave similar results except with minor details indicative of active feeding. A holding experiment with New Brunswick quahaugs gave essentially the basic lipid composition but after 10 wk compositional variations in fatty acid were suggestive of the effect of nonfeeding. The close fatty acid resemblence among several samples suggests that the ocean quahaug eventually converts ingested fatty acids to a species-specific composition based on those acids common to other northwestern Atlantic shellfish. Certain details of these fatty acid analyses suggest particulate matter sources for some ingested fatty acids. No correlation between flavor and lipid or fatty acid composition could be obtained because flavor problems were not observed in these samples.

Ackman, R. G., S. Epstein, and M. Kelleher. 1974. A comparison of lipids and fatty acids of the ocean quahaug, *Arctica islandica*, from Nova Scotia and New Brunswick. J. Fish. Res. Board Can. 31: 1803–1811.

Nous avons comparé des lots de quahaugs de mer, *Arctica islandica*, provenant respectivement de la Nouvelle-Ecosse et du Nouveau-Brunswick, dans le but de déterminer si la composition en acides gras peut refléter les sources locales de nourriture et expliquer ainsi de prétendus problèmes de saveur. Les teneurs en lipides augmentent de mai à juillet, mais une comparaison détaillée des acides gras, y compris ceux des chairs et de l'hépatopancréas, donnent des résultats semblables, sauf pour des détails mineurs, indicateurs d'une alimentation active. Une expérience de maintien en vivier de quahaugs du Nouveau-Brunswick donne essentiellement la même composition de base en lipides, mais après 10 semaines, les variations de composition en acides gras évoquent l'effet du jeûne. Le fait que les acides gras se ressemblent beaucoup dans plusieurs échantillons donne à croire que la quahaug de mer convertit éventuellement les acides gras en une composition propre à l'espèce, à base d'acides communs aux autres coquillages de l'Atlantique Nord-Ouest. Certains détails de ces analyses suggèrent que quelques-uns des acides gras ingérés proviennent de la matière particulaire. Nous n'avons pu trouver de corrélation entre la saveur et la composition en lipides ou en acides gras, parce que ces échantillons ne présentèrent pas de problèmes de saveur.

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The increasing closure of traditional Canadian Atlantic inshore shellfish sources of clams *Mya arenaria* and bay quahaugs (hard clams) (*Mercenaria mercenaria*) has focussed attention on offshore resources of shellfish, particularly the ocean quahaug *Arctica islandica* (Anon. 1971; Medcof and Chandler 1968; Parker and McRae 1970; Bissell 1972). In Nova Scotia this species was recently harvested for a few years by commercial interests operating out of Port Medway (Hazelton 1972).

Previous attempts to exploit this species had been made sporadically along the Atlantic coast of Canada and the USA, but reportedly occasional severe problems with especially strong flavors had led to the collapse of these industries (Mendelsohn et al. 1970; Bissell 1972). Flavor elements in shellfish may on occasion reflect intake of algae (Ackman

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and Hingley 1968), the process probably paralleling the accumulation of algal toxins (Bourne 1965; Prakash et al. 1972).

One of the accessible biochemical starting points for study of the influence of algae on suspected flavors in ocean quahaugs lay in the lipids and fatty acids. Accordingly, in parallel with taste panel studies (L. W. Regier unpublished data) we have compared these components in commercially caught ocean quahaugs from Port Medway, N.S., with fresh samples from Passamaquoddy Bay, N.B., and with the latter after 10 wk of live-holding. The lipid levels showed variations subject to plausible explanations, and the fatty acid variations which were recorded in these particular samples have various interpretations, including a detrital feeding pattern. No objectionable flavors were encountered, negating that part of the study.

Experimental — Live ocean quahaugs from Passamaquoddy Bay were supplied by the St. Andrews, N.B., Biological Station of the Fisheries and Marine Service. They were shipped to Halifax by air in damp seaweed and were placed in filtered running sea water (Stewart and Power 1963) within 24 h of being fished. Approximately 12 h later 20 raw quahaugs were hand-shucked for study except for half of one lot fished May 19, 1971, which were left undisturbed for 10 wk before shucking. Commercial catches from off Port Medway, N.S., were collected off incoming vessels and live-held overnight in the same tanks before shucking. Animals of uniform size (6–7 cm in length) were selected for all studies. The distinctive dark "hepatopancreas" of the shucked quahaugs was removed for study separately from the rest of the body. Lipid extraction and gas–liquid chromatographic studies were similar to those applied to oysters (Watanabe and Ackman 1974). It should be noted that identifications of some minor fatty acid components are tentative and the provision of two places of decimals does not imply a corresponding order of accuracy but is solely to show relative proportions of minor components. A shorthand notation is used for chain length: number of ethylenic bonds and position of bonds.

Results and discussion — The recovery of total lipids (Table 1) shows that 10 wk of live-holding had only a superficial effect on the gross composition of the May samples from New Brunswick. The July New Brunswick shipment appeared to contain substantially more neutral lipid in both flesh and hepatopancreas. This observation is partly based on the concurrent lower proportions of phospholipids, indicating that much of the additional lipid is neutral lipid as would be expected during a fattening period. The Nova Scotia sample (June) is very similar to the July New Brunswick sample. These results support those of Skúladóttir (1967), who reported total meat fat of Icelandic ocean quahaugs at a seasonal maximum of 2.1–2.6% in June, compared to a minimum of 0.9% in September, with May values of 1.2–1.9% indicative of increasing fat deposition compared to the lean winter period.

The fatty acid compositions of certain lipid fractions for the New Brunswick May sample as received were not considered reliable due to possible autoxidative losses of polyunsaturated fatty acids during analysis and are not included in Table 2, but valid partial data for hepatopancreas phospholipids and triglycerides are presented in Table 3. The lipids of the same lot of animals after 10 wk of live-holding under nonfeeding conditions (filtered sea water) showed about the same composition as the original lipid, but the fatty acids of the live-held

TABLE 1. *Arctica islandica* sample origin, lipid recovery, and percentage of polar lipids in lipid.

Origin	Date received 1971	Tissue	Percentage of: Lipid in fresh tissue	Percentage of: Polar lipids in lipid
N.B.	May 20	Flesh	0.34	56.2
		Hepatopancreas	1.15	62.2
" "	May 20[a]	Flesh	0.43	55.4
		Hepatopancreas	1.01	79.1
" "	July 19	Flesh	0.80	49.8
		Hepatopancreas	1.20	39.7
N.S.	June 29	Flesh	0.69	–
		Hepatopancreas	1.15	–

[a]After 10 wk live-holding.

TABLE 2A. Comparisons of fatty acids from specific flesh lipids of *A. islandica* freshly collected off Nova Scotia (June 29) and off New Brunswick either fresh (July 19) or after 10 wk live-holding (from May 20). Data in weight percent. ND = not determined, usually for technical rather than sample reasons.

	Phospholipids			Triglycerides			Sterol Esters		
		N.B.			N.B.			N.B.	
Fatty acid*	N.S. Fresh	Fresh	10 wk	N.S. Fresh	Fresh	10 wk	N.S. Fresh	Fresh	10 wk
14:0	0.37	0.37	0.64	3.53	3.91	5.28	4.19	4.02	5.89
4,8,12-TMTD	0.18	0.16	0.10	0.64	ND	4.47	1.64	1.26	2.89
Iso 15:0	0.04	0.03	ND	0.21	0.18	0.24	0.28	0.32	1.17
Anteiso 15:0	0.02	0.06	ND	0.11	0.18	0.71	0.55	1.28	3.50
15:0	0.41	0.52	0.63	0.54	0.52	1.19	0.83	2.07	1.75
Iso 16:0	0.29	0.41	0.62	0.21	0.61	1.41	0.66	1.42	ND
Pristanic	0.18	0.53	0.40	ND	ND	ND	0.43	0.61	ND
16:0	12.91	9.79	12.67	32.35	27.52	29.67	18.64	11.37	21.93
7-MHD	0.11	0.34	0.49	0.23	0.03	0.85	0.52	0.88	1.10
Iso 17:0	0.44	0.19	0.51	0.84	0.48	0.93	0.81	1.56	1.14
Anteiso 17:0	0.29	0.37	1.13	0.84	1.57	2.56	2.71	4.38	2.86
17:0	1.74	1.43	2.06	1.69	1.45	1.75	0.81	0.63	0.57
Phytanic	ND	ND	0.30	ND	ND	0.14	ND	0.15	0.28
Iso 18:0	0.36	0.73	0.66	0.63	0.72	0.63	1.88	1.32	0.71
18:0	6.26	7.02	8.76	7.93	4.06	5.25	7.79	4.33	6.89
19:0	0.21	ND	ND	0.41	1.06	0.63	0.53	0.15	0.28
20:0	ND	ND	ND	0.05	ND	0.11	ND	0.76	ND
Total saturated	23.8	21.9	29.0	50.2	42.3	55.9	42.2	36.5	51.4
14:1ω7	ND	0.05	ND	0.22	ND	ND	ND	ND	ND
15:1	ND	0.20	ND	ND	ND	ND	0.44	0.16	ND
16:1ω9	0.15	ND	0.31	0.84	ND	0.93	2.44	1.09	2.86
16:1ω7	1.74	1.99	2.47	12.64	12.78	12.71	2.71	3.13	6.29
16:1ω5	0.15	ND	0.41	ND	ND	0.47	ND	ND	ND
17:1	0.14	0.74	0.15	ND	0.48	0.35	ND	0.15	0.28
18:1ω13	2.07	2.07	3.03	0.83	0.35	0.80	1.06	1.84	0.70
18:1ω9	1.92	1.89	2.02	3.72	3.55	4.57	6.92	5.21	6.48
18:1ω7	2.85	1.95	1.61	8.26	6.85	6.28	5.32	3.22	3.05
18:1ω5	ND	0.12	0.25	0.41	0.24	0.40	ND	ND	0.10
19:1	0.14	ND	0.15	0.10	0.30	0.34	ND	0.08	1.40
20:1ω11	0.28	0.90	0.45	0.41	0.58	0.51	0.79	2.27	0.27
20:1ω9	1.06	0.90	0.70	1.69	0.94	1.41	3.95	5.69	0.65
20:1ω7	1.47	4.18	2.77	5.31	3.13	2.80	18.77	16.14	6.45
20:1ω5	ND	ND	ND	ND	ND	ND	ND	ND	ND
22:1ω11+13	ND	ND	ND	0.10	0.14	0.11	0.65	2.40	0.82
22:1ω9	ND	ND	ND	0.25	0.07	ND	1.30	1.13	0.48
22:1ω7	ND	ND	ND	0.08	ND	ND	0.65	0.75	ND
Total monoethylenic	10.5	15.0	14.3	34.1	29.4	31.7	45.0	43.3	29.8
18:2ω9	ND	0.30	2.02	ND	ND	ND	ND	ND	0.14
18:2ω6	0.32	0.18	0.46	0.36	0.59	0.46	0.93	1.00	0.96
20:2ω6	0.91	1.19	0.54	0.56	0.41	0.22	0.65	0.38	ND
(20:2)"a"	10.64	7.24	13.48	0.92	0.29	0.28	2.63	2.27	ND
(20:2)"b"	0.84	2.69	0.94	1.82	1.45	1.12	5.99	3.75	2.37
(22:2)"A"	1.85	2.69	1.23	0.66	0.52	0.22	1.63	3.00	0.41
(22:2)"B"(?)	ND	ND	ND	ND	ND	0.11	ND	ND	0.14

(*Continued*)

TABLE 2A. Comparisons of fatty acids from specific flesh lipids of *A. islandica* freshly collected off Nova Scotia (June 29) and off New Brunswick either fresh (July 19) or after 10 wk live-holding (from May 20). Data in weight percent. ND = not determined, usually for technical rather than sample reasons. — (*Concluded*)

	Phospholipids			Triglycerides			Sterol Esters		
		N.B.			N.B.			N.B.	
Fatty acid*	N.S. Fresh	Fresh	10 wk	N.S. Fresh	Fresh	10 wk	N.S. Fresh	Fresh	10 wk
Total diethylenic	14.6	14.3	18.7	4.3	3.3	3.4	11.8	10.4	4.0
18:3ω6	0.44	ND	0.05	0.03	ND	0.53	ND	0.31	0.07
18:3ω3	1.32	0.43	0.36	1.27	0.30	0.29	0.27	0.79	0.60
20:3ω6	ND	ND	ND	ND	ND	ND	ND	ND	ND
20:3ω3	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total triethylenic	1.8	0.4	0.4	1.3	0.3	0.8	0.3	1.1	0.7
18:4ω3	ND	ND	1.06	0.65	1.60	1.11	0.28	ND	0.66
20:4ω6	2.06	2.98	2.81	0.17	0.63	0.38	ND	0.17	1.09
20:4ω3	2.88	0.64	0.72	ND	0.14	ND	ND	0.45	0.62
22:4ω6	0.60	1.35	0.31	ND	ND	ND	ND	ND	ND
Total tetraethylenic	5.5	5.0	4.9	0.8	2.4	1.5	0.3	0.6	2.4
20:5ω3	15.28	15.12	15.31	5.48	16.40	6.41	0.83	1.43	1.37
21:5ω2	0.61	1.05	0.53	0.14	0.96	ND	ND	ND	0.92
22:5ω6	0.15	0.70	0.14	ND	ND	ND	ND	ND	ND
22:5ω3	2.21	2.60	1.39	0.36	0.32	ND	ND	ND	0.87
Total pentaethylenic	18.2	19.5	17.4	6.0	17.7	6.4	0.8	1.4	3.2
22:6ω3	23.91	18.85	14.55	2.75	4.71	2.00	ND	5.24	2.55
I.V. (Calc.)	231.9	217.0	192.7	85.4	134.8	78.5	75.8	99.7	80.0

*4,8,12-TMTD = 4,8,12-trimethyltridecanoic acid; pristanic = 2,6,10,14-tetramethylpentadecanoic acid; phytanic = 3,7,11,15-tetramethylhexadecanoic acid; 7-MHD = 7-methylhexadecanoic acid, the latter measured after hydrogenation of methyl esters.

quahaugs show some novel details which could be attributed to nonfeeding.

Comparison of the hepatopancreas phospholipid shows that the freshly received animals (New Brunswick, May 20) had a remarkable proportion of 4,8,12-trimethyltridecanoic (4,8,12-TMTD) acid, a phytol degradation product. The only comparable high level of this acid had been reported in feeding periwinkles (Ackman et al. 1971). The level dropped during the 10 wk of live-holding (Table 2B). It may be concluded that the May shipment of New Brunswick quahaugs had been feeding actively. Tyler (1973) shows high caloric values for *A. islandica* tissue starting in April and May, supporting this probability. The 4,8,12-TMTD itself does not necessarily point to a diet of fresh algae. The low proportion of 22:6ω3[1] in the two hepatopancreas lipids of the May quahaugs from New Brunswick (Table 3) is not due to autoxidation (note that 20:5ω3, which would be equally susceptible, is normal) and this suggests that these animals were feeding heavily on algae. Among the fatty acids of unicellular marine algae 20:5ω3 is often much more prominent than 22:6ω3 (Ackman et al. 1968).

In the flesh triglycerides (Table 2A) of fresh (July 19) New Brunswcik *A. islandica* percentages of 20:5ω3 and 22:6ω3 are more than twice what may be thought normal. Two alternative explanations may be offered. Phagocytosis, known to be important in *A. islandica* (Winter 1970), could result in all or part of algal lipids migrating with phagocytes and temporarily modifying the usual triglyceride fatty acid pattern. Mammalian phagocytes can also incorporate foreign lipid hydrolysis products such as free fatty acids or lysolecithin into their own membranes without fatty acid degradation (Elsbach 1972). Against this may be placed the belief that phagocytes are most active in the hepatopancreas

[1]Shorthand notation for fatty acids is chain length: number of ethylenic bonds, and position of ethylenic bond system relative to terminal methyl group.

TABLE 2B. Comparisons of fatty acids from specific hepatopancreas lipids of *A. islandica* freshly collected off Nova Scotia (June 29) and off New Brunswick either fresh (July 19) or after 10 wk live-holding (from May 20). Data in weight percent. ND = not determined, usually for technical rather than sample reasons.

	Phospholipids			Triglycerides			Sterol Esters		
		N.B.			N.B.			N.B.	
Fatty acid*	N.S. Fresh	Fresh	10 wk	N.S. Fresh	Fresh	10 wk	N.S. Fresh	Fresh	10 wk
14:0	1.39	1.07	1.19	4.60	3.87	4.07	7.92	8.59	6.02
4,8,12-TMTD	4.78	5.94	1.87	2.76	2.82	0.57	2.70	3.79	2.21
Iso 15:0	0.23	0.04	0.09	0.13	0.26	0.14	0.68	2.13	ND
Anteiso 15:0	ND	0.09	0.24	0.06	0.40	0.22	1.36	2.98	ND
15:0	0.88	0.62	0.94	0.47	0.66	0.65	1.71	4.68	0.75
Iso 16:0	0.38	1.05	0.70	0.20	0.62	0.78	1.18	1.26	1.85
Pristanic	0.41	0.59	0.45	ND	0.13	0.07	1.64	2.45	0.36
16:0	22.46	19.57	15.16	20.04	23.58	25.39	20.27	22.33	18.08
7-MHD	0.08	0.12	0.26	0.10	ND	0.12	ND	ND	ND
Iso 17:0	0.45	0.78	0.46	0.25	ND	1.13	0.33	0.63	1.83
Anteiso 17:0	0.15	1.64	1.85	0.12	2.08	1.69	1.34	2.92	5.85
17:0	2.25	2.60	1.73	1.61	1.82	ND	0.67	0.83	0.55
Phytanic	0.15	ND	ND	ND	ND	0.42	0.16	0.40	0.35
Iso 18:0	0.15	0.86	0.46	0.25	0.51	0.86	0.66	0.41	ND
18:0	11.60	9.00	8.58	5.03	5.01	3.64	7.78	6.40	6.51
19:0	ND	0.42	0.51	0.30	0.95	ND	2.46	0.41	0.36
20:0	0.15	0.25	0.13	0.12	ND	ND	0.97	0.40	0.35
Total saturated	45.7	44.6	34.7	36.0	42.7	41.0	51.8	60.6	45.1
14:1ω7	0.23	ND	ND	0.13	ND	0.73	1.89	ND	ND
15:1	0.27	0.35	ND	0.13	0.13	ND	1.35	1.26	ND
16:1ω9	ND	ND	0.35	0.50	ND	1.13	4.68	4.17	1.83
16:1ω7	1.20	3.12	2.08	14.38	7.78	11.86	4.35	4.17	5.48
16:1ω5	ND	ND	0.12	0.25	ND	0.56	ND	ND	ND
17:1	ND	ND	ND	0.49	0.32	0.28	ND	0.83	ND
18:1ω13	1.91	2.37	1.81	0.85	0.38	0.44	0.33	0.61	1.43
18:1ω9	3.24	1.70	2.04	2.67	3.31	3.74	9.84	6.44	6.81
18:1ω7	5.16	3.39	2.49	10.45	7.25	5.26	3.93	2.25	3.58
18:1ω5	ND	ND	0.11	0.36	0.25	0.55	ND	0.20	0.36
19:1	ND	ND	ND	0.30	0.38	ND	0.57	ND	ND
20:1ω11	0.29	0.25	0.45	0.36	0.63	0.27	0.49	0.46	1.42
20:1ω9	1.60	1.09	0.67	1.62	1.07	0.55	2.19	1.72	4.26
20:1ω7	2.60	1.45	2.11	3.39	3.55	1.22	6.43	4.11	14.39
20:1ω5	ND	ND	ND	ND	ND	ND	0.41	ND	ND
22:1ω11+13	ND	ND	ND	ND	0.06	ND	0.32	0.75	1.36
22:1ω9	ND	ND	ND	ND	0.05	ND	0.40	0.75	1.23
22:1ω7	ND	ND	ND	ND	ND	ND	ND	0.25	ND
Total monoethylenic	16.5	12.3	12.2	35.9	25.2	26.6	37.2	28.0	42.1
18:2ω9	ND	ND	0.17	ND	ND	ND	ND	ND	ND
18:2ω6	0.44	0.34	0.45	1.03	0.73	0.33	0.08	1.23	0.90
20:2ω6	1.15	0.83	0.55	0.36	0.40	0.27	0.40	ND	ND
(20:2)"a"	7.29	7.56	11.21	0.60	0.63	0.55	0.81	0.20	2.13
(20:2)"b"	1.15	1.41	1.66	1.07	1.00	1.09	1.61	1.80	3.86
(22:2)"A"	1.62	0.75	1.55	0.42	0.55	ND	0.80	0.25	0.97
(22:2)"B"(?)	ND	ND	ND	ND	ND	ND	ND	ND	2.00

(*Continued*)

TABLE 2B. Comparisons of fatty acids from specific hepatopancreas lipids of *A. islandica* freshly collected off Nova Scotia (June 29) and off New Brunswick either fresh (July 19) or after 10 wk live-holding (from May 20). Data in weight percent. ND = not determined, usually for technical rather than sample reasons. — (*Concluded*)

	Phospholipids			Triglycerides			Sterol Esters		
	N.S.	N.B.		N.S.	N.B.		N.S.	N.B.	
Fatty acid*	Fresh	Fresh	10 wk	Fresh	Fresh	10 wk	Fresh	Fresh	10 wk
Total diethylenic	11.6	10.9	15.6	3.5	3.3	2.2	3.7	3.5	9.9
18:3ω6	ND	ND	0.34	0.14	ND	ND	ND	2.10	2.39
18:3ω3	2.07	2.18	2.27	0.75	0.19	0.21	1.47	2.05	0.55
20:3ω6	ND	ND	ND	ND	ND	ND	ND	ND	ND
20:3ω3	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total triethylenic	2.1	2.2	2.6	0.9	0.2	0.2	1.5	4.1	2.9
18:4ω3	0.36	ND	0.57	2.82	1.66	ND	0.86	0.22	ND
20:4ω6	0.82	1.14	1.27	0.27	0.65	0.46	0.27	0.11	ND
20:4ω3	3.49	3.02	4.13	0.22	ND	ND	1.36	ND	ND
22:4ω6	0.12	ND	0.24	ND	ND	ND	ND	ND	ND
Total tetraethylenic	4.8	4.2	6.2	3.3	2.3	0.5	2.8	0.3	–
20:5ω3	9.56	13.75	15.54	20.09	20.13	22.45	0.87	0.64	ND
21:5ω2	0.36	0.22	0.24	0.40	0.69	ND	0.16	0.34	ND
22:5ω6	0.08	ND	0.25	ND	ND	ND	ND	ND	ND
22:5ω3	1.01	ND	1.36	ND	0.43	ND	1.02	ND	ND
Total pentaethylenic	11.0	14.0	17.4	20.5	21.2	22.4	2.0	0.9	–
22:6ω3	8.39	9.66	11.61	ND	5.07	5.05	0.75	ND	ND
I.V. (Calc.)	135.7	149.3	179.9	137.9	146.3	144.2	66.5	50.3	68.2

*4,8,12-TMTD = 4,8,12-trimethyltridecanoic acid; pristanic = 2,6,10,14-tetramethylpentadecanoic acid; phytanic = 3,7,11,15-tetramethylhexadecanoic acid; 7-MHD = 7-methylhexadecanoic acid, the latter measured after hydrogenation of methyl esters.

and the effect might be expected to be observed there, which it is not (Table 2B). However, in *Mytilus edulis* the hepatopancreas reportedly has a temporary storage function (Gabbott and Bayne 1973), and in several molluscs the digestive gland accumulates paralytic shellfish poison. The crystalline style lipase of the surf clam *Spisula solidissima* has recently been shown to be ineffective against 20:5ω3 and 22:6ω3 (Patton and Quinn 1973). Therefore, it is also possible that the monoglyceride resulting from this effect, if applicable in *A. islandica*, would be speedily reesterified with fatty acids and transferred to the flesh as part of the process of triglyceride accumulation during heavy feeding. After 10 wk of nonfeeding this flesh triglyceride was apparently converted to the species-specific composition, probably by turnover of local cellular lipid.

The fatty acids of the flesh lipids (Table 2A) unfortunately would also include those accumulated for gonad development. These may differ from muscle fatty acids in some details. Although flesh phospholipid 20:5ω3 is remarkably uniform in the three samples, 22:6ω3 may be ranked as: Nova Scotia, June 29> New Brunswick, July 19> New Brunswick, May 20, 10 wk. Referring to the hepatopancreas phospholipids one finds that percentages for both 20:5ω3 and 22:6ω3 show a reverse ranking order (Table 2B). The figures for Σ20:5ω3 + Σ22:6ω3 for the three samples, in the above order, are 57, 57, and 58%. Although possibly only a coincidence, this suggests that the two tissue phospholipid compositions are kept in some sort of balance in respect to these two related fatty acids. Fatty acid alteration activity, particularly in the hepatic phospholipids, may be indicated by the accumulation of 20:4ω3, usually ≤0.5% in marine lipids.

The hepatopancreas triglycerides, unlike the phospholipids, probably show a nearly constant composition for the two acids (Tables 2B and 3).

TABLE 3. Some fatty acids of hepatopancreas phospholipids and triglycerides of *A. islandica* from New Brunswick (May 20) as received. Data in weight percent.

Fatty acid	Phospholipids	Triglycerides
14:0	1.7	4.7
4,8,12-TMTD*	9.6	2.7
16:0	17.2	15.5
18:0	9.4	4.4
Total saturated‡	46.3	38.0
16:1ω7	2.5	10.9
18:1ω13	0.9	0.6
18:1ω9	2.9	4.1
18:1ω7	3.3	6.8
20:1ω11	0.7	0.6
20:1ω9	1.0	1.6
20:1ω7	2.9	2.7
Total monoethylenic‡	15.6	29.7
(20:2) "a"	5.6	0.4
(20:2) "b"	0.9	0.9
20:4ω3	3.8	0.3
20:5ω3	13.6	21.2
22:6ω3	6.8	3.6

*4,8,12-trimethyltridecanoic acid.

‡From complete analysis.

The flesh triglycerides are probably more variable because they act as temporary storage for surplus fatty acids such as 20:5ω3 and 22:6ω3 (see New Brunswick, July 19, Table 2A, and agreement from the high total flesh lipid (Table 1) for these quahaugs. Proportionately 20:5ω3 in this triglyceride is three times the percentage of the other two flesh triglycerides, but 22:6ω3 is only twice as much, indicating as suggested above that 20:5ω3 is of direct algal origin, whereas 22:6ω3 may be of algal origin or be partially converted from 20:5ω3. Visceral triglycerides of the Japanese prickly scallop *Chlamys nipponensis akazara* also show relatively less 22:6ω3 than flesh triglycerides (Hayashi and Yamada 1973), implying a storage function for this acid in the latter lipid.

The proportions of NMID (20:2) "a" (nonmethylene-interrupted diethylenic C_{20} acids; "a" is probably the Δ^5, Δ^{11} isomer; "b" the Δ^5, Δ^{13} isomer; (22:2) A and B are the related C_{22} acids discussed by Ackman and Hooper 1973) are noticeably high in both the flesh and hepatopancreas phospholipids of the live-held animals. It has been proposed that in marine molluscs this acid is a replacement for "essential" fatty acids of the "ω6" or linoleic acid family (Ackman and Hooper 1973). The percentages for saturated acids in the three flesh lipids of these animals also tended to be higher than in the other two lots of quahaugs which were analyzed shortly after being fished, and this could also reflect de novo fatty acid biosynthesis during nonfeeding.

In the absence of dietary unsaturated fatty acids the ocean quahaug may also exploit the route 16:0 → 16:1ω7→ 18:1ω7→ 20:1ω7 to supply cellular needs for unsaturated fatty acids. The high proportions of ω7 to ω9 acids in the C_{18} and C_{20} chain lengths of all samples (Table 2) are most unusual even for marine animals (cf. Ackman and Hooper 1973; Watanabe and Ackman 1974).

The presence of a suspected 18;1ω13 fatty acid as a high proportion (>18:ω9) in 18:1 acids of phospholipids and sterol esters is unusual. This relatively unknown acid is of much less significance in periwinkles (*Littorina littorea*) and moon snails (*Lunatia triseriata*), although basically related to the NMID acids as discussed by Ackman and Hooper (1973). Certain microorganisms are possible sources of 18:1ω13 (Quint and Fulco 1973) but primitive plants and marine algae cannot be excluded from consideration involving Δ^5 ethylenic unsaturation (Jamieson and Reid 1972).

In most molluscs such as oysters *Crassostrea virginica* or bay quahaugs (*Mercenaria mercenaria*) (Ackman et al. 1971) as well as the periwinkle and moon snail, the rule is percent *iso* > percent *anteiso* for both the C_{15} and C_{17} acids. It is therefore unexpected to see *anteiso* > *iso* in some of the lipids of the ocean quahaug. This is a subject worthy of further study with isolation and further detailed study as they could be linked to diet. It has recently been suggested that these acids may be particularly associated with surface lipids (van der Horst et al. 1973). In ocean quahaugs the low level of α-tocopherol (Ackman and Cormier 1966), and the absence of dimethyl-β-propiothetin (Ackman and Hingley 1967), both imply that the diet is low in intact unicellular algae. Therefore, the diet could be largely detritus as the ocean quahaugs live on relatively clean clay or in firm mud bottoms at depths of 10–60 m (C. Medcof personal communication), or somewhat deeper than the other species that we have examined for fatty acids and which have access to the surface waters richer in unicellular phytoplankters. Potentially the higher ratio of *anteiso* to *iso* acids in the ocean quahaug could reflect marine bacteria, free or attached to the detritus surface, although only a few species of bacteria are known to emphasize *anteiso* branching (Bowie et al. 1972; Sen Gupta 1972). Alternatively an accumulation of these acids synthesized de novo from appropriate amino acid precursor would give comparable results (Kaneda 1973; Sen Gupta 1972). In the absence of explicit information on diet, dissolved nutrients may also be suggested as a factor in establishing this fatty acid ratio (Testerman 1972).

The accumulation of 4,8,12-TMTD (4,8,12-trimethyltridecanoic) acid, a degradation product of phytol origin, is not unusual in mollusc lipids (Ackman et al. 1971). However, in this study the greater association with hepatopancreas phospholipids and triglycerides than with the corresponding flesh lipids is new information. In contrast the measurement of 7-MHD (7-methylhexadecanoic) acid in the hydrogenated samples showed that either it or the various isomeric 7-methyl-hexadecanoic acid precursors (Ackman et al. 1973) were associated to a greater extent with the flesh lipids.

The reasonable consistency of overall fatty acid patterns as well as details suggests that three analyses of ocean quahaugs show a species-oriented fatty acid compositon rather than a diet-oriented composition, although it is not known if this would apply to animals from other than the Canadian Atlantic coastal shelf. The relevant literature and discussion will be found elsewhere (Watanabe and Ackman 1974).

Conclusions on flavor problems — The taste panels run on quahaugs in conjunction with the lipid studies reported a consistent strong flavor, but no specific lot of quahaugs could be shown to have a serious objectionable flavor (L. W. Regier personal communication).

The results of this survey of spring and summer samples show that lipids in the ocean quahaug are not normally apparently subject to local influences other than those imposed by the seasonal and reproductive patterns noted by Skúladóttir (1967). The fatty acids show probable temporal and spatial variations between the Pasamaquoddy Bay–Nova Scotia shelf sites but tend to revert to a species-specific composition with nonfeeding live-holding. Some fatty acid details imply that intact live algae may not be the major dietary factor common in shallow water filter-feeding molluscs, but others support such a diet in fattening animals. This suggests that the reports of strong flavors occurring in specific times or locations may not always be immediately associated with local surface events such as algal blooms. If such flavors were of exogenous origin, then naturally occurring detrital substances of complex structure such as pacifenol (Sims et al. 1973) could have antibiotic activity and suppress decomposition of algal remnants by bacteria. The ingestion of this detritus by ocean quahaugs would permit accumulation of these same unusual molecules in a fashion similar to that of biotoxins (Bourne 1965) or dimethyl-β-propiothetin (Ackman and Hingley 1967). Phagocytosis in *A. islandica* is effective at low food concentrations and is also a nonselective process (Winter 1970). Flavor components could also be accumulated from the dissolved state (Southward and Southward 1972; Testerman 1972 Péquignat 1973). Accumulated free amino acids (probably from the diet, or assimilated from sea water) are also potential flavor components and are reportedly more significant in molluscs in a stressed condition in polluted areas (Jeffries 1972, e.g. in the bay quahaugs *M. mercenaria*). The ocean quahaug occurs in restricted areas where other shellfish are uncommon, and often in beds of uniform-sized animals, suggesting that breeding is only occasionally successful and that nutritive conditions may often be borderline for this species. It is not known if this form of stress parallels the effects arising from a polluted environment (cf. mussels *M. edulis*, Gabbott and Bayne 1973).

The descriptions of the strong flavor usually refer to it as "iodine-like." This suggests to the chemist certain classes of chemical compounds, and it is therefore of interest that periostracum is believed to be made up of "quinone tanned protein" (Fox 1973) The growth of *A. islandica* is probably irregular and seasonal being from June to September in Passamaquoddy Bay (C. Medcof personal communication), and any output of "quinones" in the generative epithelial cells described by Bubel (1973) for periostracum fixation would be concentrated in an area where it would be readily detected by man. If our samples did not coincide with this period it would account for the fact that we have been unable to locate a specific lot of quahaugs having the flavors described in the literature.

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Some Details of Fatty Acids and Alcohols in the Lipids of North Atlantic Copepods

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ACKMAN, R. G., B. A. LINKE, AND J. HINGLEY. 1974. Some details of fatty acids and alcohols in the lipids of North Atlantic copepods. J. Fish. Res. Board Can. 31: 1812–1818.

Fatty alcohols and fatty acids from both triglycerides and wax esters of a mixed plankton sample consisting mostly of the copepods *Centropages typicus*, with some *Calanus finmarchicus*, and a few other organisms, were compared. The alcohols were probably derived from both calanoids and emphasized alc 20:1 (19.2%) and alc 22:1 (44.9%) despite the virtual absence of the corresponding monoethylenic fatty acids. The isomer composition for monoethylenic alcohols parallels that observed for acids in clupeid depot fats and it is suggested that this particular wax ester composition is a result of low density lipids, especially alc 22:1, being deposited during heavy feeding to offset the mass from codeposition of protein and carbohydrate.

ACKMAN, R. G., B. A. LINKE, AND J. HINGLEY. 1974. Some details of fatty acids and alcohols in the lipids of North Atlantic copepods. J. Fish. Res. Board Can. 31: 1812–1818.

Nous comparons les alcools et les acides gras des glycérides aussi bien que des esters de cire d'un échantillon de plancton mixte, comprenant surtout le copépode *Centropages typicus*, un certain nombre de *Calanus finmarchicus* et quelques autres organismes. Les alcools sont probablement dérivés des deux calanoïdes et sont surtout alc 20:1 (19.2%) et alc 22:1 (44.9%), malgré l'absence presque totale d'acides gras monoéthyléniques correspondants. La composition isomère des alcools monoéthyléniques est parallèle à celle observée dans les acides des dépôts de graisse des clupéidés, et nous suggérons que cette composition particulière de l'ester de cire résulte de lipides de faible densité, surtout alc 22:1, qui seraient déposés au moment où les organismes se nourrissent abondamment, dans le but de compenser la masse contre une déposition concomitante de protéines et de carbohydrates.

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WAX esters are found to be the dominant class of lipids in many planktonic crustaceans. It has been postulated that they serve as long-term energy stores. However, triglycerides are also important in most of the planktonic crustaceans known to have wax esters, and in some comparable crustaceans triglycerides are the only major neutral lipid (Lambertsen and Myklestad 1971; Lee et al. 1971a, 1971b, 1972; Morris 1972; Yamada 1972; Gatten and Sargent 1973; Morris and Sargent 1973; Sargent et al. 1974). Most gas–liquid chromatographic studies have accounted for the major component fatty acids in the triglycerides and wax esters, and for the corresponding fatty alcohols in the latter. Investigations of certain minor components, such as methyl-branched fatty acids, and of most isomers of monoethylenic fatty acids of various chain lengths have not been reported.

Our earlier study of fatty acids of *Temora longicornis* by open-tubular gas–liquid chromatography indicated a much wider pattern of fatty acids than reported elsewhere (Ackman and Hooper 1970). We have therefore surveyed a mixed planktonic copepod crustacean sample from the North Atlantic near Nova Scotia for minor fatty acids and alcohols, and for details of isomers in monoethylenic acids and alcohols occurring as major components. The sample was dominated numerically by *Centropages typicus*, but accompanied by a high proportion of *Calanus finmarchicus* which is known to be rich in wax esters. We propose that a requirement for a low-density lipid may be the reason for the particular long-chain monoethylenic alcohols found.

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Materials and methods — The sample was collected in a plankton net towed by the M.V. *Pandalus II* at 3–15 m depth, at lat. 44°26′30″N, long. 63°25′30″W, on October 22, 1973, and was observed to be very rich in small crustaceans. The sample was brought to the laboratory in cold sea water and held in cold sea water overnight prior to removal of detritus, plant remains, large fish eggs, and a few large animals. Sample filtration gave a recovery of 216 g of animals, many alive, and superficially homogeneous as to size, color etc., from which the species composition (Table 1) was determined on a random 16-g sample preserved in formalin.

The rest (200 g) yielded 1.2% of lipid when immediately extracted by the method of Bligh and Dyer (1959). The lipid was streaked on Prekote Adsorbosil-5 (Applied Science Laboratories) silica gel thin-layer chromatography plates and developed in hexane: diethyl ether: acetic acid: 85:15:1. An exhaustive series of extractions of recovered lipid bands with ethyl ether: $CHCl_3$::1:1 gave lipid recoveries (based on material applied) of: wax esters 64.6%, triglycerides 6.0%, and fatty acids 3.3%. The recovered triglycerides and wax esters were transesterified with BF_3-MeOH. The methyl esters of the triglycerides were then recovered and analyzed by gas–liquid chromatography without further purification. The whole wax ester transesterification product was recovered and separated by thin-layer chromatography into methyl esters and free fatty alcohols. The latter were converted to acetate esters for gas–liquid chromatographic study by refluxing with acetic anhydride.

In addition to examination of esters as recovered, parts of all ester samples were completely hydrogenated and the product examined quantitatively and qualitatively by gas–liquid chromatography. The latter procedure included separate analyses on two open-tubular columns, 46 m in length × 0.25 mm ID, coated, respectively, with butanediol-succinate polyester or SILAR-5CP (Applied Science Laboratories), in Perkin-Elmer series 900 apparatus. Data is presented to two decimal places to permit inclusion of minor components and does not imply this order of accuracy. Major components should be accurate to ±5% and moderate-sized components to ±10%, but components ≤1% may be subject to larger relative errors.

TABLE 1. Numerical percentage composition of part of the October 1973 plankton sample studied for lipids.

Species	Percentage
Centropages typicus	78
Calanus finmarchicus	17
Euphausiacea (Juveniles)	2
Temora sp.	1
Cladocera sp.	1
Metridia longa	0.3
Fish eggs	0.2
Spiratella retroversa	0.1
Tortanus discaudatus	0.1

Results and discussion — The lipid recovery of 1.2% (wet weight) from this plankton sample is comparable to the 1.3–1.7% obtained for three surface species of copepods by Morris (1971; 1972). Unfortunately most other reports of lipid content are on a dry weight basis, but extreme variability for either dry or wet weight analyses is not unusual among many calanoid copepods (Lee et al. 1971a; Gatten and Sargent 1973), or wax ester-rich mysids such as *Gnathophausia* sp. (0.74–4.74%, wet weight; Morris and Sargent 1973). The moderate total fat could result from the known absence of the oil droplet in *C. typicus* (M. Paranjape personal communication) which is prominent in many calanoid copepods (see *C. finmarchicus* figure in Lee et al. 1972). If an omnivore (Petipa et al. 1970), *C. typicus* would perhaps have less need for depot fats than would carnivores with specific dietary needs. *Centropages* sp. taken near Vancouver were reported to include only 2% triglyceride and 9% wax ester in total lipid (Lee and Hirota 1973) with the balance presumably phospholipid, normally about 0.5% of muscle weight. The level of lipid recovered from our sample is thus not unreasonable for the major copepod species of our sample. Estimating from approximately equal lipid levels in *C. finmarchicus*, with 80% wax ester (Gatten and Sargent 1973), and in *C. typicus* with 10% wax ester, the wax ester recovered should be of the order of two thirds from *C. finmarchicus* and one third from *C. typicus* to yield the found percentages of lipids. As lipid of other origins (e.g. fish eggs) may have altered the analytical results in minor ways this must be regarded as a very approximate basis for discussion.

The fatty acids of the triglycerides and of the wax esters (Table 2) are comparably complex and correspond qualitatively to those found in diverse lower marine animals such as the sea anemone *Metridium dianthus* (Hooper and Ackman 1971) or euphausiids *Meganyctiphanes norvegica* (Ackman et al. 1970), and also in higher marine organisms such as the white barracudina *Paralepis rissoi krøyeri* Bonaparte 1840 (Ackman et al. 1972) or the ocean sunfish *Mola mola* (Hooper et al. 1973). The details of minor saturated and unsaturated fatty acids, and of different isomers in the monoethylenic fatty acids, supplement the major components listed for copepod fatty acids by several authors (cited herein). The complexity of the fatty acid mixture is probably common to most copepods as these details resemble many in the fatty acids of a small sample of *T. longicornis* (Ackman and Hooper 1970). The latter appears to include fatty acids from

TABLE 2. Detailed composition of fatty acids from triglycerides, and fatty acids and alcohols from wax esters (in weight percent) from mixed copepod plankton sample.

Fatty acid[a]	Fatty acids: Triglyceride	Fatty acids: Wax ester	Fatty alcohols: Wax ester
12:0	0.06	0.25	0.06
13:0	0.01	0.20	Trace
Iso 14:0	0.01	0.14	0.05
14:0	6.27	32.17	2.01
4,8,12-TMTD	0.06	2.24	0.02
Iso 15:0	0.31	0.48	0.16
Anteiso 15:0	0.17	0.28	0.08
15:0	1.11	2.09	0.40
Iso 16:0	0.10	0.13	0.12
Anteiso 16:0	0.02	0.01	ND
16:0	25.49	11.27	12.62
7-MHD[b]	0.10	0.04	Trace
2,6,10,14-TMPD	0.03	0.02	0.05
Iso 17:0	0.23	0.14	0.16
Anteiso 17:0	0.11	0.05	0.03
17:0	0.96	0.23	0.16
3,7,11,15-TMHD	0.01	0.07	0.02
Iso 18:0	0.24	0.08	0.18
Anteiso 18.0?	Trace	0.02	ND
18:0	3.02	0.28	0.43
Unknown	0.42	Trace	ND
19:0	0.04	0.01	0.05
20:0	0.15	0.04	0.44
21:0	Trace	NIL	ND
22:0	0.12	Trace	0.07
24:0	ND	ND	0.33
Saturated	38.9	50.2	17.3
14:1ω7	0.02	0.19	NIL
14:1ω5	0.05	0.23	Trace
16:1ω11	NIL	NIL	ND
16:1ω9	0.57	0.37	Trace
16:1ω7	2.96	15.62	5.47
16:1ω5	0.24	0.30	0.27
17:1ω8	0.21	0.19	0.28
17:1ω6	0.05	0.03	0.04
18:1ω13	NIL	NIL	ND
18:1ω11	NIL	NIL	NIL
18:1ω9	3.63	3.28	3.23
18:1ω7	2.51	0.64	1.84
18:1ω6?	0.10	0.05	0.03
18:1ω5	1.23	0.14	0.34
19:1ω8?	0.03	Trace	0.04
20:1ω11	0.13	0.23	1.48
20:1ω9	0.38	0.83	17.20
20:1ω7	0.11	0.09	0.57
20:1ω5	0.03	0.02	0.15
22:1ω13+11	Trace	Trace	38.99
22:1ω9	Trace	ND	5.40
22:1ω7	ND	ND	0.51
22:1ω5	ND	ND	0.20
24:1ω9	ND	ND	0.45
ΣMonoene	12.2	22.2	76.5
16:2ω6	0.02	0.46	ND
16:2ω4	0.23	1.52	ND
18:2ω4?	0.05	0.02	ND
18:2ω6	1.15	0.55	1.46
20:2ω6	0.17	0.07	0.20
22:2ω6	ND	ND	0.01
ΣDiene	1.6	2.6	1.7
16:3ω4?	0.10	2.12	ND
16:3ω3	Trace	0.03	ND
18:3ω6	0.05	0.19	0.07
18:3ω3	0.74	0.59	0.81
20:3ω6	0.11	0.06	0.20
20:3ω3	0.17	0.05	0.14
ΣTriene	1.2	3.0	1.2
16:4ω1?	0.02	2.87	ND
18:4ω3	0.78	7.44	0.01
18:4ω1?	0.05	0.53	ND
20:4ω6	1.19	0.34	0.09
20:4ω3	0.36	0.61	0.17
22:4ω6	0.21	Trace	ND
ΣTetraene	2.6	11.8	0.3
20:5ω3	14.12	8.59	0.07
21:5ω2?	0.20	0.10	0.16
22:5ω6	0.24	Trace	0.10
22:5ω3	0.28	0.04	0.33
ΣPentaene	14.8	8.7	0.7
21:6?	0.87	Trace	Trace
22:6ω3	27.04	0.48	1.99
Calc. IV	208	111	78

(*Continued*)

[a]4,8,12-TMTD= 4,8,12-trimethyltridecanoic acid; 2,6,10,14-TMPD(pristanic)= 2,6,10,14-tetramethylpentadecanoic acid; 3,7,11,15-TMHD(phytanic)= 3,7,11,15-tetramethylhexadecanoic acid.

[b]In hydrogenated sample, 7-methylhexadecanoic acid.

both triglycerides and wax esters. Among other acids,[1] percentages for 14:0, 16:0, 16:1, 18:0, 18:1 (approximately), 18:4ω3, 20:1, 20:4ω6, 20:5ω3, and 22:6ω3 in the *T. longicornis* analysis are all intermediate to those now found separately for the fatty acids of the triglyceride and of the wax esters presumably derived from both *C. typicus* and *C. finmarchicus*. In greater detail, the ratios in the lipids of *T. longicornis* for the pairs of isomers 16:1ω7 and 16:1ω5, and 18:1ω9 and 18:1ω7, are also evidently derived from contributions from both lipid types. This suggests that the fresh sample of *T. longicornis* studied earlier (from Placentia Bay, Nfld., collected in early June, 1969; Ackman and Hooper 1970) had essentially the same ratio of triglyceride to wax ester as the October 1973 plankton sample from the Nova Scotian shelf. Moreover the fatty acid compositions of the two lipids in *T. longicornis* must have been approximately the same as in the latter.

The fatty alcohols for the mixed copepod sample of this study (Table 2) are of considerable interest because of the major proportions of long chain-length monoethylenic materials (notably alc 20:1 and alc 22:1, plus the rarer alc 24:1). A rather similar fatty alcohol composition has been reported for *C. finmarchicus* (Lambertsen and Myklestad 1971), and *Calanus plumchrus* (Lee et al. 1972). In other copepods, e.g. *Gaussia princeps*, the fatty alcohols follow the more commonplace biochemical pattern of being dominated by alc 16:0 (Nevenzel 1970; Hooper and Ackman 1971; Lambertsen and Myklestad 1971; Ackman et al. 1971, 1972; Lee et al. 1972; Yamada 1972; Morris and Sargent 1973; cf. Rahn et al. 1973). The polyunsaturated fatty alcohols alc 20:5ω3 and alc 22:6ω3 corresponding to the major polyunsaturated fatty acids have been reported in some of the other analyses of copepod wax esters, (e.g. Lee et al. 1971a, 1971b; Morris and Sargent 1973). Clearly, many of the fatty alcohols such as the polyunsaturated forms alc 18:2ω6, alc 18:3ω3, alc 20:5ω3, and alc 22:6ω3, and the methyl-branched saturated alcohols, are derived from the corresponding precursor fatty acids, probably through intermediates such as phospholipids where the polyunsaturated acids are normal (Nevenzel 1970; Lee et al. 1971b; Patton et al. 1972; Farkas et al. 1973). The fundamental acid/alcohol interchange would lead to alcohols related to the minor saturated acids such as the *iso* and *anteiso* series.

[1]Shorthand notation for fatty acids is chain length: number of ethylenic bonds, and position of ethylenic bond system relative to terminal methyl group. The prefix alc indicates a fatty alcohol.

Although the basic biochemistry is probably the same, the few fatty alcohol analyses in the literature which show details of monoethylenic isomers have little relevence to copepods (see below). The widely held view that monoethylenic fatty acids are formed by desaturation at the ninth and tenth carbon atoms counted from the carboxyl group (Ackman and Castell 1966) must be considered in any discussion of fatty alcohols. Considering the facility of interchange (Rahn et al. 1973), it is likely that this desaturation takes place in the molecule as the acid. The different proportions of isomeric monoethylenic fatty acids show that the conversion 16:1ω7 →18:1ω7 is more important in the triglyceride than in the wax ester fatty acids, or that these two lipids use different proportions of dietary and endogenous acids. The corresponding fatty alcohols, alc 16:1 and alc 18:1, are, respectively, intermediate in isomer ratios, suggesting that both triglyceride and wax ester fatty acids are sources for alcohols, and that this conversion of monoethylenic acid into alcohol occurs independently of 16:1 or 18:1 isomer composition detail. Chain elongation of (unspecified) 18:1ω9 to give alc 20:1ω9 is plausible because alc 20:1ω9 and alc 20:1ω11 are directly identifiable from gas–liquid chromatography, and the alc 20:1ω11 isomer formed via the direct desaturation route from (unspecified) 20:0 is minor in proportion. The extension process for the ω9 molecule must further account for the considerable proportion of alc 22:1ω9. The two isomers 22:1ω13 and 22:1ω11 of fatty alcohols or acids cannot be separated by gas chromatography alone. In the 22:1 fatty acids of fish oils the ω13 isomer formed from 22:0 is normally less than the ω11 isomer formed by chain elongation from 20:1ω11 (Ackman and Castell 1966). Our results for alc 22:1 isomers suggest that the process (unspecified) 20:1ω11→ alc 22:1ω11 is distinctly probable for the fatty alcohols. The low proportion of ω11 isomer in the 20:1 alcohols need not contraindicate the possibility of this process as the proportions for alc 20:1ω11 and alc 20:1ω9 are normal for many fish depot fatty acids (Ackman and Castell 1966) where the same desaturation–elongation process of acids must occur even though 20:0 acid is a minor ($\leq 0.5\%$) component and 22:0 is seldom readily detected. The origin of alc 22:1ω11 from an (unspecified) 20:1ω11 precursor could involve desaturation, chain elongation, and alcohol formation occurring more or less as one integrated process (see discussion of polyunsaturated alcohols below).

A parallel study, based on similar analytical technology, for barracudina lipids (Ackman et al. 1972) showed that 20:1 and 22:1 fatty acids are somewhat more important components of most

barracudina lipids than in the copepods. However, in the fatty alcohols the alc 20:1 and the alc 22:1 proportions are less in barracudina than in the copepods. Notwithstanding this, the isomer distribution for barracudina alc 20:1 (0.1:7.2:0.9 for ω11, ω9 and ω7) and for alc 22:1 (4.5:0.7:0.3 for ω13+11, ω9 and ω7) are remarkably similar to those in the copepods (Table 2), implying a common basic biochemical process or source. Winterized sperm whale oil has isomer proportions of 20:75:5 for ω11, ω9, and ω7 20:1 alcohols, 50:50 for 20:1 acids, and 17:66:17 for ω13, ω11, and ω9 22:1 acids (Spencer and Tallent 1973), which further illustrates the basic similarity of the biochemical processes among mammals, fish, and crustacea involving longer-chain fatty acids and fatty alcohols (see review by Nevenzel 1970; also Farkas et al. 1973).

It has been proposed that the chain elongation of monoethylenic fatty acids in herring is a means to maximize buoyancy because of the lower specific gravity of monoethylenic fatty acids, especially 22:1 (Ackman and Eaton 1970), as compared to shorter chain lengths. The same hypothesis may apply equally in copepods. The deposition of wax esters as an energy store presumably takes place when food is freely available and protein and other nonlipid materials are also being accumulated. Their higher density could be offset by biosynthesis of suitable low-density lipid such as wax esters generally (density about 0.86 vs. 0.92 for triglycerides; Lewis 1970) or more particularly by those including alc 22:1 (cf. acid data cited by Ackman and Eaton 1970). Lee et al. (1971a) have reviewed depth, latitude, and other factors and reported preferential utilization of triglycerides compared to wax esters by starved *G. princeps*. Gatten and Sargent (1973) considered the depth aspects of lipid, wax ester content, and wax ester biosynthesis in copepods (*C. finmarchicus*). The latter group found less lipid and more wax ester biosynthetic activity in animals at the ocean surface. This could support the buoyancy–food deposition theory. The extent of published speculation implies that a unique answer is lacking. We suggest that an economical way of controlling buoyancy and accumulating a long-term energy reserve has been combined by many oceanic animals (Benson and Lee 1972; Yamada 1972; Sargent et al. 1974; Herring 1973; Kayama and Nevenzel 1974). Otherwise it is difficult to explain the need for alc 20:1 and alc 22:1 in lieu of alc 18:1 or even alc 16:0. The presence of the unusual 24:1 in copepods, as high as 7.9% of fatty alcohols in one case (Lee et al. 1971a), and especially alc 24:0, is a major indication of the extent of chain extension activity.

The presence of small proportions of polyunsaturated fatty alcohols, including those of C_{20} and C_{22} chain lengths, is not novel. For example alc 22:6ω3 has been reported for *C. helgolandicus* (Lee et al. 1971b; Lambertsen and Myklestad 1971), *Euphausia pacifica* (Yamada 1972), and *Gaetanus brevicornis* (Lee et al. 1971a, 1972). Interconversion of alcohols and acids is accepted as basic to wax ester metabolism (Lee et al. 1971b; Morris 1972; Morris and Sargent 1973; Rahn et al. 1973; Sargent et al. 1974). Recently it has been shown that polyunsaturated acids in the rat gastrointestinal tract are not readily reduced to alcohols (Bandi and Mangold 1973), a further fundamental process possibly accounting for their relatively low levels in copepod alcohols, if the biochemical analogy may be extended that far.

The virtual absence of alc 20:5ω3, relative to alc 22:6ω3, in our samples of wax esters, despite the 20:5ω3 in the acids (Table 2), is supported by the other analyses (cf. also Patton et al. 1972). The activity in chain extension biotransformations would tend to lead to more alc 22:6ω3 than alc 20:5ω3 and agrees with the monoethylenic alcohol isomer distribution formation based on the hypothesis of chain elongation and alcohol function occurring simultaneously. The high proportions reported for alc 20:4ω6 in *C. helgolandicus* (Lee et al. 1971b, 1972) seem to be more likely attributable to alc 22:1 (Table 2; see also Lambertsen and Myklestad 1971.)

The substrate specificity study in the gastrointestinal tract of the rat also showed that saturated acids and alcohols were rapidly interconverted, but monounsaturated acids less rapidly (Bandi and Mangold 1973). The occurrence of *iso*, *anteiso*, and odd-chain, normal saturated structures in the copepod alcohols (Table 2) is thus easily rationalized as part of a free interchange process. A chain-length effect blocking the conversion of shorter chain ($<C_{16}$) acids to alcohols must be operative, perhaps because of the emphasis on longer chain length, low density, alcohol formation.

In the barracudina, 14:0 was an important component acid in the triglycerides (25.8%), but in our copepod lipid sample the wax ester acids were the important deposit for this acid (32.2% versus 6.3%, in the triglycerides). This difference is further reflected in the alc 16:0 proportion. The 42% alc 16:0 in the barracudina probably is interrelated to the 14:0 acid from the (alcohol + acid) pool. In our particular copepods 14:0 was left to accumulate as wax ester acid because the synthesis of alc 16:0 may have been bypassed in favor of the postulated synthetic routes through longer chain acids such as 18:0→18:1→20:1→alc 20:1 and 20:0→20:1→22:1 →alc 22:1. Also the total chain length of the two moieties in marine wax esters usually is 34, or 36, with some 38. The 14:0 acid may be prominent to keep the wax ester near the optimum total chain

length in the presence of the longer alc 20:1 and alc 22:1. As the 20:1 acids are very minor components and the 22:1 acids are barely detectable it may be that these acids are converted to the corresponding alcohols as fast as they form. Alternatively if longer chain-length mitigates against a direct depot acid origin for the long chain alcohols (cf. Bandi and Mangold 1973), then the process of elongation could take place from appropriate alcohols via a temporary acid form.

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Parasite Fauna of Kokanee Salmon (*Oncorhynchus nerka*) Introduced into Lake Huron[1]

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In a 7-yr study, the most common parasites of kokanee salmon (*Oncorhynchus nerka*) introduced to Lake Huron were the acanthocephalans *Metechinorhynchus salmonis* (in 61% of 228 adult fish examined) and *Acanthocephalus jacksoni* (15%) and the nematode *Cystidicola stigmatura* (41%). These also occurred in fish species netted along with kokanee, but many common parasites of other salmonids in Lake Huron were not harbored by kokanee. In addition to evidence of attack by sea lamprey (*Petromyzon marinus*), 18 species of parasites were found, 13 of which had not been previously recorded for kokanee in the Pacific drainages of North America. The high incidence of *M. salmonis* and *C. stigmatura* indicates that amphipods are commonly ingested by kokanee.

COLLINS, J. J., AND A. O. DECHTIAR. 1974. Parasite fauna of kokanee salmon (*Oncorhynchus nerka*) introduced into Lake Huron. J. Fish. Res. Board Can. 31: 1818–1821.

Au cours d'une étude de 7 années, les parasites les plus communs trouvés chez des kokanis (*Oncorhynchus nerka*) introduits dans le lac Huron sont les acanthocéphales *Metechinorhynchus salmonis* (dans 61% des 228 poissons adultes examinés) et *Acanthocephala jacksoni* (15%), et le nématode *Cystidicola stigmatura* (41%). Ces parasites se rencontrent également dans les espèces de poissons capturés dans les filets en même temps que le kokani, mais plusieurs parasites communs d'autres salmonidés du lac Huron ne se trouvent pas chez le kokani. Sans compter les marques d'attaque par la lamproie marine (*Petromyzon marinus*), nous avons trouvé 18 espèces de parasites, dont 13 n'avaient pas encore été signalées chez le kokani du versant du Pacifique de l'Amérique du Nord. La forte occurrence de *M. salmonis* et de *C. stigmatura* indique que le kokani se nourrit communément d'amphipodes.

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THE introduction of kokanee salmon (*Oncorhynchus nerka*) into Lake Huron from stocks in the Pacific drainages of North America (Ricker and Loftus 1968; Collins 1971) provided an opportunity to study the parasites acquired by a new host in the fish community. From 1966 to 1972, about 290 adult and young-of-the-year fish were examined to determine what parasites other than blood protozoans were present and how they compared with those of kokanee from donor regions and with other species of fish in Lake Huron. New information on the diet of Lake Huron kokanee can be inferred from the occurrence of certain intestinal parasites, as demonstrated for other species by Margolis (1965).

Materials and methods — During the study, 228 adult kokanee and 60 young-of-the-year (fry and fingerlings) were examined for all external and internal parasites except blood protozoans. Adult fish (9, 93, 37, 36, 1, 15, 37 in the various years) were captured by angling, in experimental nets and weirs fished by the Ministry of Natural Resources and in commercial nets. Specimens were collected from widely distributed sites, including the southern shore and tributaries of Georgian Bay, the North Channel, South Bay, and the southern shore of Manitoulin Island. Specimens were examined fresh or after freezing and thawing. Fry and fingerlings were taken in fyke nets set in Blue Jay Creek in 1968 and were preserved in a 5% formalin solution. Intensity of infection was measured by counting the parasites of each host. Several species of fish

TABLE 1. Incidence of parasites (%) in 228 adult kokanee salmon (*Oncorhynchus nerka*) from Lake Huron during 1966–72, intensity of infection,[a] organs infected, and intermediate hosts.

Parasite	% fish infected	Infection intensity	Organs infected	Intermediate hosts
		Fungi		
Saprolegnia sp.	0.9	L–M	skin	none
		Trematoda		
Tetracotyle intermedia[b, c]	4.4	L–M	heart	gastropods
Crepidostomum farionis[c]	2.6	L–M	pyloric caeca	gastropods & mayflies
Diplostomulum spathaceum[b]	2.2	L–M	eye	gastropods
		Cestoda		
Triaenophorus nodulosus[b]	0.9	L	liver	copepods
		Nematoda		
Cystidicola stigmatura	40.9	L–M	swimbladder	amphipods
Rhabdochona cascadilla[c]	4.0	L	intestine	mayfly nymphs
Spinitectus gracilis	3.5	L–M	"	mayfly nymphs
Capillaria catenata	1.4	L	"	–
		Acanthocephala		
Metechinorhynchus salmonis	61.4	L–H	"	amphipods
Acanthocephalus jacksoni	14.9	L–M	"	isopods
Leptorhynchoides thecatus	1.8	L	"	amphipods
Neoechinorhynchus tumidum	1.3	L	"	ostracods
Neoechinorhynchus rutili[c]	1.3	L	"	ostracods
Neoechinorhynchus pungitius	1.3	L	"	–
Pomphorhynchus bulbocolli	0.9	L	"	amphipods
		Arthropoda		
Ergasilus caeruleus[c]	0.9	L	gills	none
Hydrachna sp.[b]	0.9	L	"	none

[a]L (light) represents 1–9 parasites per host, M (medium), 10–49 and H (heavy), 50 or more.
[b]References to these parasites in table and text are to larval stages.
[c]Parasites recorded for kokanee in Pacific drainages by Bangham and Adams (1954) or Hoffman (1967); all others listed are new records.

found in association with kokanee (i.e. captured with kokanee in nets) in South Bay (Table 2) were also examined for parasites.

The digeneans, acanthocephalans, and cestodes were stained with Mayer's carmalum or Gomori's trichrome, cleared in beechwood creosote and mounted in Piccolyte. Nematodes (cleared in lactophenol) and crustaceans (cleared in glycerine) were examined unmounted.

Data on kokanee diet were derived from 176 stomachs containing food which were examined subsequently to those reported by Collins (1971).

Parasites found and biological implications — We found 18 parasitic species belonging to six higher taxa (Table 1). The predominant parasites were the acanthocephalans *Metechinorhynchus salmonis* (in 61% of the adult fish) and *Acanthocephalus jacksoni* (15%) and the nematode *Cystidicola stigmatura* (41%). All other species occurred in less than 5% of the adults, and 79% of the adults harbored at least one species of parasite. In young-of-the-year fish, the only parasite found was a single nematode larva. No protozoans or monogeneans were found. Wounds attributed to sea lamprey (*Petromyzon marinus*) were found on 2.6% of adult kokanee.

The parasite fauna differed markedly from that of kokanee in the Pacific drainages of North America. Only five out of 18 species recorded for western kokanee (Bangham and Adams 1954; Hoffman 1967) were found in fish introduced to Lake Huron: *Tetracotyle intermedia*, *Crepidostomum farionis*, *Rhabdochona cascadilla*, *Neoechinorhynchus rutili* and *Ergasilus caeruleus*. These parasites occurred with low frequency and the number per host did not exceed 49.

The most common acanthocephalans in kokanee, *Metechinorhynchus salmonis* and *Acanthocephalus jacksoni*, and the larval trematodes *Tetracotyle intermedia* and *Diplostomulum spathaceum* were found in almost all species of fish associated with kokanee (Table 2). The less common acanthocephalans *Leptorhynchoides thecatus* and *Neoechinorhynchus* spp., the nematode *Capillaria catenata* and the mite *Hydrachna* sp. occurred in only a few of the associated fish.

Kokanee salmon were not infected by the common parasites of coregonines and other salmonids in Lake Huron, which according to Bangham (1955) were the cestodes *Triaenophorus crassus*, *Cianthocephalus truncatus*, *Proteocephalus exiguus*, and

TABLE 2. Occurrence of parasites of kokanee salmon in major associated fish in South Bay 1966–72.

Parasites of kokanee	Alewife (50)[a,b]	Rainbow smelt (86)	Yellow perch (81)	White sucker (62)	Trout-perch (61)	Splake (37)	Lake whitefish (39)	No. of species parasitized
Saprolegnia sp.	+[c]	+	+	–	–	–	–	3
Tetracotyle intermedia	+	+	+	+	+	+	+	7
Crepidostomum farionus	–	–	+	–	–	+	+	3
Diplostomulum spathaceum	+	+	+	+	+	+	+	7
Triaenophorus nodulosus	–	–	+	+	+	–	–	3
Cystidicola stigmatura	–	+	–	–	–	+	+	3
Rhabdochona cascadilla	–	–	+	+	+	–	–	3
Spinitectus gracilis	–	–	+	–	+	+	+	4
Capillaria catenata	–	+	–	–	–	–	–	1
Metechinorhynchus salmonis	+	+	+	+	+	+	+	7
Acanthocephalus jacksoni	+	+	+	+	–	+	+	6
Leptorhynchoides thecatus	–	–	+	+	–	–	–	2
Neoechinorhynchus tumidum	–	–	–	–	–	–	+	1
N. rutili	–	+	+	–	–	–	–	2
N. pungitius	–	+	–	–	–	–	–	1
Pomphorhynchus bulbocolli	–	–	–	+	+	+	–	3
Ergasilus caeruleus	–	–	+	+	+	+	+	5
Hydrachna sp.	+	–	–	–	–	–	–	1
Total	6	9	12	9	8	9	9	

[a]The scientific names from left to right are: *Alosa pseudoharengus*, *Osmerus mordax*, *Perca flavescens*, *Catostomus commersoni*, *Percopsis omiscomaycus*, *Salvelinus fontinalis* × *S. namaycush*, and *Coregonus clupeaformis*.

[b]Numerals are numbers examined.

[c]Species marked with plus + were infected and those with – were not.

Diphyllobothrium spp.; the copepods *Salmincola inermis* and *Actheres coregoni*; and the monogenean *Discocotyle sagittata*; and according to A. O. Dechtiar (unpublished data) the cestode *Eubothrium salvelini* and the monogenean *Tetraonchus variabilis*.

The high incidence of *Metechinorhynchus salmonis* and *Cystidicola stigmatura* for which the intermediate hosts are *Pontoporeia affinis* and *Anisogammarus* spp. (DeGuisti and Budd 1959; Brownell 1970; Mamayev 1971; Bauer and Nicol'skaya 1952), indicates that amphipods are a common food item of kokanee. Since we found *P. affinis* in less than 1% of the kokanee stomachs sampled during July and August, we conclude that amphipods are taken at other times of the year. The presence of mayflies (Ephemeroptera) in about 7% of the kokanee stomachs is consistent with the occurrence of the nematode *Rhabdochona cascadilla*, for which the intermediate hosts are nymphs of mayflies (Gustafson 1939).

Certain parasites we found in kokanee have created problems elsewhere. *Metechinorhynchus salmonis* which infected 61% of the fish with heavy intensity of infection in some, is detrimental to the nutrition of the host when it is abundant (Petrushevski and Kogteva 1954). Kokanee were only lightly infected with other pathogenic forms which are prevalent in indigenous fish of Lake Huron. These parasites, *Pomphorhynchus bulbocolli*, *Triaenophorus nodulosus*, and *Tetracotyle intermedia*, occur in about 50% of the white sucker (*Catostomus commersoni*), northern pike (*Esox lucius*), and lake whitefish (*Coregonus clupeaformis*), respectively, (A. O. Dechtiar unpublished data).

Kokanee were not abundant in Lake Huron during their initial years of introduction. There has been no indication to date that an epizootic might occur in response to the adaptation of this new host to the ecosystem. None of the parasites recorded thus far are harmful to humans or would affect the palatability of fish flesh.

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Fish Kill Due to "Cold Shock" in Lake Wabamun, Alberta

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ASH, G. R., N. R. CHYMKO, AND D. N. GALLUP. 1974. Fish kill due to "cold shock" in Lake Wabamun, Alberta. J. Fish. Res. Board Can. 31: 1822–1824.

A thermal fish kill due to "cold shock" caused by a mechanical failure in the steam electric generating unit was observed in Lake Wabamun, Alberta on February 8, 1973 in the region of the discharge canal. The most rapid temperature decline occurred in the initial 30 min after shutdown when the temperature dropped 16.9 C (21.8 to 4.9 C). The major fish species affected included spottail shiners (*Notropis hudsonius*) and northern pike (*Esox lucius*). The freshwater clam *Anodonta* sp. did not appear to be affected.

ASH, G. R., N. R. CHYMKO, AND D. N. GALLUP. 1974. Fish kill due to "cold shock" in Lake Wabamun, Alberta. J. Fish. Res. Board Can. 31: 1822–1824.

Une mortalité de poissons due à un "choc au froid", causé par une panne du générateur électrique à vapeur, a été observée dans le lac Wabamun, Alberta, le 8 février 1973, dans la région du canal de déversement. L'abaissement de température le plus rapide s'est produit au cours des 30 minutes qui ont suivi l'arrêt du générateur, alors que la température a baissé de 16.9 C (21.8 à 4.9 C). Les principales espèces de poisson affectées comprenaient le queue à tache noire (*Notropis hudsonius*) et le grand brochet (*Esox lucius*). La coque d'eau douce *Anodonta* sp. n'a pas semblé affectée.

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MOST literature on biological effects of thermal discharges has dealt with sudden temperature increases, that is, "heat shock." However, in temperate regions sudden decreases in temperature, or "cold shock," may be of greater importance, particularly during the winter. This aspect of thermal pollution was noted by Alabaster (1969) but has not been documented with the exception of a few notes appearing in bulletins (Anon. 1971, 1972). It is obvious that "cold shock" effects upon various organisms must be studied in greater detail since Cairns (1972) in his recommendations for the protection of aquatic communities from thermal shock states that "fish and other aquatic organisms shall not be acclimated to temperatures so high that they would die from low temperature stress if the (power) plant were to cease functioning in midwinter."

A "cold shock" fish kill was observed in Lake Wabamun, Alberta in 1973. Lake Wabamun is located approximately 64 km (40 miles) west of Edmonton, Alta. and presently has two fossil fuel steam electrical generating plants which utilize the lake for cooling purposes. The Sundance plant (300 megawatts [MWe] capacity from two units at the time of the fish kill) and the Wabamun plant (600 MWe capacity from two units) are located on the south and north shores, respectively. Detailed morphometry as well as the physical, chemical, and biological properties of the lake are given in Nursall and Gallup (1971), Gallup and Hickman (1973), and Gallup and Hickman (in press).

In the early morning of February 8 the one operating unit at the Sundance plant was forced to shut down due to mechanical failure. In 30 min the water temperature dropped 16.9 C in the discharge canal. For discussion the canal was divided into three sections: upper, middle, and lower (Fig. 1). The middle section had an experimental system of 24 modules that sprayed a portion of the discharge water into the atmosphere for cooling. A fabric curtain served to channel the water to the sprayers. At the time of shutdown the discharge of the canal was approximately 284 m^3/min (60,000 imperial gal/min).

Study area and methods — By February 11 sufficient ice to support the investigators had formed on the lower portion of the discharge canal and the area was examined to determine the effects of the rapid temperature decline. During the shutdown there was insufficient ice formation on the middle and upper portions of the discharge canal to allow examination. These portions were examined on February 14 after the plant had resumed operation and the ice on the canal had melted. An estimate of the numbers and species of fishes killed here was made by visual counts from a boat.

Five transects of nine holes each were established across the lower portion of the canal (Fig. 1). Seven

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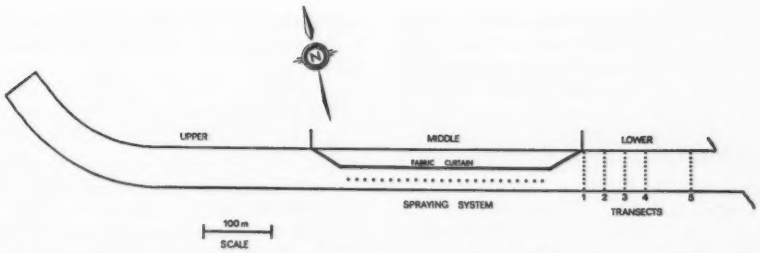

FIG. 1. Physical layout of the discharge canal. The position of the transects of holes cut in the ice is shown in the lower portion of the canal.

of the nine holes were evenly spaced at 6.5-m intervals across the 52-m-wide canal. The remaining two holes were located 1 m from each shore. Each hole was 1 m². The first transect was at the lower end of the spraying system. Transects 2, 3, 4, and 5 were 30 m, 60 m, 90 m, and 150 m, respectively, downstream from Transect 1.

The number and the species of dead fish lying on the substrate immediately below each hole was recorded. The maximum depth in the canal was approximately 2 m and the water was clear enabling this assessment to be accurate. Samples of the dead fish were collected and weighed to determine their biomass.

The number of fish counted under each hole was taken to represent the average number of fish per square meter for the area one-half the distance to the neighboring holes and one-half the distance to the neighboring transects. To estimate the total number of fish killed in the study area, the numbers for all the areas were summed. This procedure was applied only to spottail shiners (*Notropis hudsonius* Clinton) since the fishes observed were almost exclusively this species.

Results and discussion — Temperature decline after plant shutdown was rapid. Data obtained from a temperature recorder located at the upper end of the discharge canal showed that the greatest temperature decline occurred in the initial 30 min after shutdown when the temperature dropped 16.9 C (21.8 to 4.9 C) (Fig. 2). From then on the decline was more gradual until the canal eventually froze over.

In the transect area established on February 11 there were an estimated 158,000 dead spottail shiners. The mean number of dead spottails per square meter was 16.4 with a range of 0–200. Only six yellow perch (*Perca flavescens*) were counted in the area and no total estimate was made. The average wet weight of a spottail shiner as determined from a sample of 100 fish was 1.32 g. Thus, the biomass of dead spottail shiners in this area was estimated to be 209 kg (364 lb).

Observations from the boat on February 14 revealed large concentrations of dead spottail shiners around the sprayers. This probably was because the sprayers remained in operation after the shutdown; thus the water currents moving towards the intakes tended to concentrate dead fish in the area. An estimate of 100,000 dead spottail shiners was made for the middle portion of the canal. Only a few dead spottail shiners were observed in the upper portion of the canal above the sprayers.

In about one-half of the canal, 122 dead northern pike (*Esox lucius*) were counted from the boat. An estimate of 250 dead pike was made for the entire canal. The average weight of 10 individuals collected was 730 g; therefore, the total biomass of dead pike was estimated to be approximately 182 kg. In addition one small (15 cm) burbot (*Lota lota*) and three small (20–25 cm) white suckers (*Catostomus commersoni*) were collected. However, only dead spottail shiners and northern pike were found in large numbers, probably due to their greater utilization of the discharge canal.

In summary, approximately 258,000 spottail shiners (341 kg = 750 lb) and 250 northern pike (182.5 kg = 402 lb) were killed in the discharge canal. Unfortunately, it is not possible to assess the impact of this fish kill upon the total lake system since population estimates of fish in the lake are not available.

No dead lake whitefish (*Coregonus clupeaformis*), which are the mainstay of a commercial and sports fishery on the lake, were observed in the canal. It was

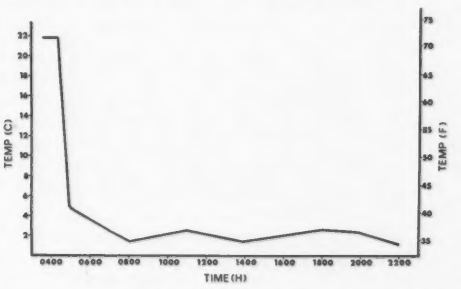

FIG. 2. Temperature of the cooling water discharge before and after shutdown on February 8, 1973.

also noted that the freshwater clam (*Anodonta* sp.), which is abundant in the canal, did not seem to be affected by the rapid temperature decline. Carmine dye placed on the gills of clams taken from the canal showed that the animals had good ciliary movement on the gills. Also, specimens that were brought back to the laboratory and placed in aquaria did not exhibit adverse effects.

To guard against future fish kills of this type, certain precautions should be taken. First, power plants should consist of a number of independent generating units. This would ensure that if one unit experienced mechanical difficulties and was forced to shut down, the other units would continue to discharge hot water at or near the same temperature. Also, if plants shut down for routine maintenance, they should phase down slowly, thereby gradually cooling the discharge water and allowing the fish to become acclimated to the cooler water.

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Effect of Feeding Brown Trout (*Salmo trutta*) a Diet Pelleted in Dry and Moist Forms

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POSTON, H. A. 1974. Effect of feeding brown trout (*Salmo trutta*) a diet pelleted in dry and moist forms. J. Fish. Res. Board Can. 31: 1824–1826.

Growth rate, body composition, and intake of either dry matter or energy did not differ significantly ($P > 0.05$) between two groups of juvenile brown trout (*Salmo trutta*) fed either a low- or high-moisture semipurified diet at different rates to compensate for a diet energy density gradient. Feed/gain efficiency, on an as-fed basis, was significantly greater ($P < 0.01$) in fish fed the low-moisture diet, but did not differ ($P > 0.05$) when compared on the basis of dry matter or energy intake.

POSTON, H. A. 1974. Effect of feeding brown trout (*Salmo trutta*) a diet pelleted in dry and moist forms. J. Fish. Res. Board Can. 31: 1824–1826.

Le rythme de croissance, la composition du corps et l'entrée de matière sèche ou d'énergie ne diffèrent pas de façon significative ($P > 0.05$) entre deux groupes de jeunes truites brunes (*Salmo trutta*) soumis à un régime semi-purifié, soit à basse soit à haute teneur en eau, et à des taux différents, afin de compenser un gradient de densité d'énergie dans les deux régimes. L'efficacité nourriture/gain, comparée sur base du régime tel quel, est nettement plus grande ($P < 0.01$) chez les poissons soumis au régime à faible teneur en eau, mais ne diffère pas ($P > 0.05$), lorsque comparée sur base d'entrée de matière sèche ou d'énergie.

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DURING the past two decades the use of pelleted feeds containing intermediate and low levels of moisture (30 and <10%, respectively) has become widespread on a production basis (Crawford et al. 1973). Experimental diets, however, usually have a high moisture content of 50% or more (Wolf 1951; Halver 1969). Such high-moisture experimental diets require refrigeration or frequent preparation to protect labile nutrients. Fungal growth is a problem in prolonged holding of either intermediate- or high-moisture diets (Crawford et al. 1973). The use of low-moisture experimental diets should circumvent many problems associated with high-moisture diets. However, a paucity of published information exists on the effect of level of dietary moisture on growth and efficiency of feed utilization by salmonids. Therefore, this study was designed to determine the effects of varying the water level of a pelleted experimental diet on the growth, body composition, and efficiency of feed utilization of juvenile brown trout (*Salmo trutta*).

Materials and methods — The experimental diet (Table 1) was fed in moist- and dry-pelleted forms. The dry-pelleted diet was processed with steam in a commercial pellet mill, crushed to an acceptable feeding size (1.5–3.0 mm diameter), and refrigerated at 5 C until fed. The moist pellet was prepared by adding 100 g of tap water/100 g of dry diet according to a modification of the procedure of Wolf (1951). After the diet solidified at 5 C refrigeration, cut slices were ground in an auger-type food grinder and extruded through a plate with 2–3-mm diameter perforations. Following additional refrigeration to obtain a consistency more amenable to breaking, the extruded food strands were broken into edible-sized particles and refrigerated at 5 C until fed. This diet was prepared at 2-wk intervals.

Duplicate samples of each diet were dried at 90 C for 5 h to determine water content. The dry- and moist-pelleted diets contained 9.6 and 55.0% water, respectively. Total available calories were calculated according to the method of Phillips and Brockway (1959). The diet contained 354 kcal/100 g of dry matter. Thus, the dry- and moist-pelleted diets contained 320 and 160 kcal of available energy per 100 g, respectively. The available energy density of the low-moisture diet was either above or within the range of dietary energy levels reported in other salmonid nutritional studies (Fowler et al. 1969; Luquet 1971).

Duplicate lots of 250 brown trout each, with an initial mean body weight of 6.6 g, were fed experimental diets for 17 wk. The dry and moist diets were fed at 1.5 and 3.0% of body weight/day on an as is basis and at 1.36 and 1.35% of body weight/day on a dry basis (Norris et al. 1973; Windell et al. 1969). Groups of fish were reared at 8.3 C in circular tanks having a capacity of 150 liters and a water flow of 7.6 liters/min. All fish were fed 4 times daily, and were weighed every other week and at the end of the experiment. Daily records were maintained on mortality.

Carcass samples of 10 fish per lot were taken at the end of 17 wk and analyzed for protein, fat, ash, and water content. Significance of differences was established by Student's *t* test.

Results and discussion — Fish fed the moist diet grew slightly faster than those fed the dry diet

TABLE 1. Composition of pelleted low-moisture diet.[a]

Ingredient	% of total dry ingredients
Vitamin-free casein[b]	54
Gelatin[b]	4
Mineral mix[b,c,d]	4
Glucose[b]	18
Arginine·HCl	2
CMC[e]	2
Vitamin mix[f]	10
Corn oil[g]	6
Total	100

[a]High-moisture diet contained 100 ml of supplemental water/100 g dry diet.

[b]Casein, gelatin, salt mineral mix, and glucose were purchased from Nutritional Biochemical Corp., Cleveland, Ohio.

[c]Salt mineral mixture No. 2 (USP XIII) was supplemented with the following (in milligrams/1.00 g of salt mixture): $AlCl_3$, 18; $ZnSO_4$, 352; $CuSO_4$, 507; $MnSO_4$, 80; KI, 17; and $COCl_2$, 105.

[d]Salt mixture No. 2 (USP XIII) composition is described in Pharmacopoeia of the United States of America. Thirteenth Revision, (USP XIII). 1947. p. 721.

[e]Carboxymethylcellulose gum (medium viscosity).

[f]Vitamins added to furnish the following (in milligrams or IU/100 g dry diet): thiamine, 5; riboflavin, 20; pyridoxine HCl, 5; choline chloride, 500; niacin, 75; calcium pantothenate, 50; inositol, 200; biotin, 5; folic acid, 1.5; ascorbic acid, 100; B_{12}, 0.01; menadione sodium bisulfite, 4; dl-alpha-tocopherol acetate, 50; retinyl palmitate (water dispersable vitamin A palmitate), 400 IU; and ergocalciferol (water dispersable vitamin D_2), 1000 IU. Each 10 g of vitamin mixture contained approximately 9 g of cellulose.

[g]Tenox R (kindly furnished by W. J. Simcox, Eastman Chemical Products, Inc.) was added as an antioxidant to corn oil at 0.1% of dry diet.

TABLE 2. Effect of diet moisture on growth, food utilization, and body composition of brown trout (*Salmo trutta*).

Form of pelleted diet[a]	Final body wt[b] (g)	Feed/gain			Body composition			
		As fed (g/g)	Dry matter (g/g)	Energy[c] (kcal/g)	H_2O (%)	Protein (%)	Fat (%)	Ash (%)
Dry	18.9	1.4	1.2	4.4	72.4	17.4	5.5	2.8
Moist	21.4	2.6	1.2	4.1	72.8	17.6	5.4	2.6
Pooled SEM	0.60	0.01	0.04	0.10	0.04	0.07	0.11	0.09
P[d]	NS	0.01	NS	NS	NS	NS	NS	NS

[a]Dry and moist diets contained 9.6 and 55.0% water, respectively. See Table 1 for complete composition.
[b]Mean body weight at start was 6.6 g.
[c]kcal/g gain = feed/gain (as fed) × available kcal/g feed.
[d]P = probability level at which diet effects are significant. NS = not significant (0.05).

(Table 2) but the difference was not significant ($P > 0.05$). There was no significant difference in carcass moisture, protein, fat, and ash content of the fish fed the experimental diets ($P > 0.05$). Trout fed the moist diet consumed almost twice as much feed/gram of gain as those fed the dry diet ($P < 0.01$) on an as-fed basis. However, on a dry-matter basis, consumption of feed/gram of gain was not different ($P > 0.05$) for the two diets. Overall feed per gain efficiency as measured by the consumption of available dietary kilogram-calories per gram of gain, although slightly higher in fish fed the moist diet, was not significantly ($P > 0.05$) affected by diet.

The two diets were readily accepted when the fish were fed 4 times/day. Consumption of dry matter of the two diets was similar ($P > 0.05$). Total mortality was less than 0.5% in each dietary group.

This study showed that the trout fed diets containing 9.6 and 55% moisture at similar rates on a dry-matter basis grew at nearly the same rate and had similar feed conversions. The response of other salmonid species to level of dietary moisture was not examined in this study. However, unpublished studies by R. R. Smith, U.S. Fish and Wildlife Service, Western Fish Nutrition Laboratory, Hagerman, Idaho, indicate that either rainbow trout (*Salmo gairdneri*) or coho salmon (*Oncorhynchus kisutch*) grew equally well regardless of level of dietary water, but that chinook salmon (*Oncorhynchus tshawytscha*) grew fastest when the diet contained 35% water. He found that the water content of the ingesta within the stomach after these three species of fish were fed identical diets was highest in rainbow trout, intermediate in coho salmon, and lowest in chinook salmon. The possible inverse relationship between the optimal level of dietary water required by a specific salmonid and the capability of the fish to incorporate environmental water into its ingesta merits further study.

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PERSPECTIVES

Optimization of the salmonid nursery stream

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MUNDIE, J. H. 1974. Optimization of the salmonid nursery stream. J. Fish. Res. Board Can. 31: 1827–1837.

The minimum unit of the environment containing the essential processes of smolt production is identified as a riffle–pool sequence at a meander. Optimization for smolt rearing is seen to lie through control of discharge, temperature, food production, and cover. The pathways of food production are traced. Ways of increasing the stock of fish-food organisms include adjustment of the ratio of riffle to pool area, choice of streamside vegetation, control of light to the stream, and inorganic and organic enrichment. Ways of making food available to fry include collecting drifting invertebrates at night and releasing them in the day, dislodgement of benthos, attracting aerial insects by lights, and supplementary feeding with artificial foods. None of these procedures, when applied to a natural stream, seems economically feasible. When applied, however, to channels made alongside streams and stocked by the parent stream, they should combine some desirable features of rivers with the productive capacity, but not the costs, of hatcheries.

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Nous avons trouvé que l'unité minimale d'environnement qui renferme les processus essentiels de production de smolts est une succession radier–fosse dans un méandre. On se rend compte que l'optimisation de l'élevage de smolts se produit par contrôle de l'émissaire, de la température, de la production de nourriture et de l'abri. Nous traçons le cheminement de la production alimentaire. Parmi les moyens d'augmenter le stock d'organismes mangés par les poissons, on note le réglage du rapport de superficie des radiers à celle des fosses, le choix de la végétation riveraine, le contrôle de la lumière sur le cours d'eau et l'enrichissement inorganique et organique. Les façons de rendre la nourriture accessible aux alevins incluent la cueillette nocturne d'invertébrés avec remise en liberté le jour, le délogement du benthos, l'attraction des insectes ailés à l'aide de lumières et l'alimentation supplémentaire avec de la nourriture artificielle. Aucune de ces procédures, lorsque appliquées à un cours d'eau naturel, ne semble économiquement rentable. Par contre, lorsque appliquées à des chenaux aménagés le long de et alimentés par le cours d'eau parent, ces méthodes devraient combiner certaines caractéristiques désirables des rivières avec la capacité productrice, mais non les coûts, des établissements de pisciculture.

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THE purpose of this paper is to examine briefly the stream ecosystem to see which of its features might lend themselves to economically feasible manipulations which would increase the production of salmon smolts. No exhaustive review of the literature is attempted; a critique is offered of the more obvious factors limiting production and of ways by which they might be altered. The treatment covers salient principles rather than distinctions between different species and different streams. It is necessary to consider the whole ecosystem, because of the interdependence of its parts. By optimization is meant: "making conditions most favorable," in this case for underyearling salmon; stream conditions could, of course, be adjusted to benefit other organisms.

The view is taken that our understanding is usually insufficient to let us make even rough quantitative predictions of the results of deliberate environmental change, yet the direction — or sign — of the result should be predictable, and should be positive if a sound decision has been made. Again, because of the limited field experimentation which has been carried out on salmon streams, it

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is not possible, in working towards optima, to evoke the sophisticated control methods used in engineering and business, although these might have application in ecology (see, for example, Dale 1970). The analysis, therefore, can only be elementary; it owes much to recent syntheses of knowledge of river mechanics (Leopold et al. 1964), river biology (Hynes 1970a), and stream salmonids (Northcote 1969; White and Brynildson 1967).

The Environmental Unit

The distribution of juvenile salmonids in different streams may be related directly to the migratory behavior and choice of spawning areas of the adults. The most favorable juvenile rearing habitat, however, may contrast with the sites chosen by the spawners, and may not be accessible to newly emerged fry. The distinction between spawning and rearing requirements can be seen within a stream. Chinook fry (*Oncorhynchus tshawytscha*), for example, emerge from the large gravel of Campbell River on Vancouver Island, B.C., which has a typical flow of 12 m^3/s, and move to slack water at the extreme edge of the river to feed; presumably most of the river does not provide suitable feeding territory for very small fry. Coho fry (*O. kisutch*) in smaller rivers show the same behavior (Mundie 1969; Lister and Genoe 1970), and size-related changes in rearing requirements have been noted for Atlantic salmon (*Salmo salar*) (McCrimmon 1954, Saunders and Gee 1964), which enter areas of stronger current and coarser bottom as they grow. Irrespective of the requirements of 1+ and older fish it appears, then, that small streams are most suitable for underyearling salmonids (see McCrimmon 1954 for the fate of underyearling planted salmon in main streams).

It is not obvious why large rivers tend to produce fewer numbers of salmon fry, in terms of numbers per unit area, than small ones. Slopes being equal, large rivers flow faster than small ones, and their generally higher velocities may be detrimental to fry. Other factors, however, are involved. Large rivers have higher discharges — river width tends to be proportional to the square root of the dominant discharge (Kellerhals 1967) — and discharge, being proportional to velocity and depth has some correlation with tractive forces on the river bed (Leopold et al. 1964). The amount of scour of organic materials and benthic organisms is likely to be greater, therefore, in large rivers than in small ones. An exception is provided by large sand rivers, which absorb much of the tractive force in the formation of dunes and ripples.

An exception to low productivity in large rivers is provided by stretches of river below lake outflows; here production may be high because of seston originating from the lakes.

Since small streams can be highly productive, and since they offer the three major types of flowing water habitat — riffles, pools, and intermediate reaches — they will serve for an examination of rearing properties.

A first question is: what unit of the stream contains all the principles and processes relevant to the production of salmon? Is it necessary to deal with the whole stream? In high-gradient streams a single riffle–pool sequence at a meander can be interpreted as the basic environmental unit. This sequence is a consequence of vertical waves being formed when water flows downhill over geological and soil materials of different sizes. The spacing of riffles and pools tends to be regular, as was first noted by a salmon biologist (Stuart 1953), and their formation can be explained in terms of the least rate of potential energy expenditure per unit mass of water, within constraints applied largely by substrate erodibility and roughness (Yang 1971). A corollary is the dispersion and sorting of bed materials, with the substrata of riffles acquiring their large particles — boulders and cobbles — near the surface (Fig. 1). These are the sites of high intergranular flows and of dispersal of invertebrates which become available to fry as organic drift.

In addition to vertical waves other waves produce horizontal sinuosities. These are most conspicuous as meanders in low gradient streams and they also occur with regularity. Here again sorting of bed material results, and also the formation of undercut banks (Fig. 1c), frequently a conspicuous feature of the salmon habitat.

If a natural channel is made smooth, the flows, in time, will create riffles and pools; if one is straightened, they will bend it into S-shaped curves. Vertical and lateral undulations of the physical environment are therefore fundamental properties of the flowing water ecosystem with biological significance comparable, say, to the formation of thermoclines in lakes.

A riffle–pool sequence can be regarded, then, as the minimal unit of the environment which need be considered; a stream is essentially a repetition of such units.

Discharge, because it has *some* correlation with fluid stress, and because it affects area of submerged bed is, of course, the overriding influence, and the extremes of flow — freshets (spates) and droughts — are recognized as major determinants of fish production (e.g. Smoker 1953; Onodera and Ueno 1961; Havey and Davis 1970). Within the

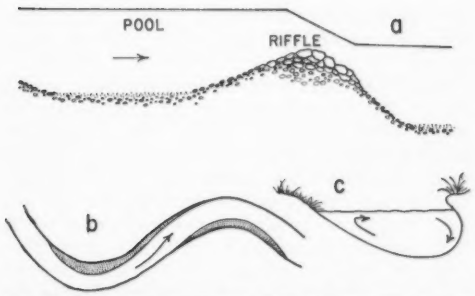

FIG. 1. The environmental unit of streams: a, the riffle–pool sequence; b, meander with areas of deposition shaded; and with undercut banks, c, on the outside of the bend.

extremes, however, it is difficult to demonstrate the influence of discharge (see Havey 1974 for Atlantic salmon). Ideally, therefore, the first decision in optimizing a nursery stream would be to set discharge within defined limits. This control would not aim at uniform annual flow, for above-average discharges, in alluvial streams, determine, among other things, the size-distribution of the substrate materials, and therefore the amount of fines in the bed, and of cobbles and boulders which give essential summer and winter cover for fish (McCrimmon 1954; Bjornn 1971). Unfortunately year-round flow control of entire streams is scarcely ever a practical possibility, and even where it is feasible, on account of a special watershed morphometry, it is very costly. Partial and local control measures are exemplified by stream-improvement devices and habitat alterations (e.g. Saunders and Smith 1962; Hunt 1971 for *Salvelinus fontinalis*). These can be applied, of course, only where annual freshets are insufficient to undo them (Ehlers 1956).

The influence of temperature is also fundamental. Most emphasis has been placed on upper lethal temperatures, how they can develop in small streams when riparian vegetation is removed, and how they can be prevented (e.g. Brown and Krygier 1970). It has been pointed out, however, that trees can be felled along cold, densely shaded, streams to improve temperatures for salmon (Burns 1972). In this way substantial changes can be made which will affect not only the metabolism of fry and parr but the rates of all productive processes. A doubling of the number of generations of common species of food organisms might result (Hynes 1970a), and, if adequate amounts of food were available, the duration of stream life of salmonids might prove to be adjustable. A cautionary mention of the dangers of lowering winter temperatures by removal of forest cover is necessary.

Although the warming of small streams seems to have valuable potential, it is plain that the consequences of excessive temperatures are likely to be far more serious than those of suboptimum temperatures. The geographic range of stream salmonids in North America is probably being reduced by high temperatures resulting from deforestation (Hynes 1970b).

The Food Web in Relation to Salmon

The wide array of items in the diet of salmon fry and parr is well known (e.g. see Maitland 1965; Egglishaw 1967; Thomas 1962 for Atlantic salmon; Mundie 1969 for coho; Becker 1973 for chinook). To understand their sources the food web must be considered. In detail this is perhaps unimaginably complex; in broad principle major strands are recognizable (Fig. 2). Optimization of the ecosystem should strengthen the ones which lead to salmon and suppress others.

In general streams are mainly heterotrophic producers, perhaps two-thirds of what is produced being dependent on decomposing terrestrial plant material and dissolved organic matter. Aquatic autotrophs — algae, mosses, and angiosperms — although they may be locally abundant are frequently too sensitive to the instability of the substrate, and to low light intensities, to form the major basis for production.

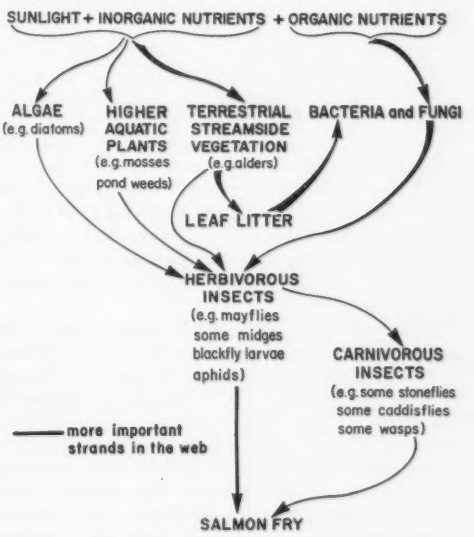

FIG. 2. Major strands in the food web leading to salmon.

The decomposing organic materials have particulate and dissolved fractions. Aquatic insects may, therefore, ingest algae, leafy fragments, large and small particles of detritus with associated microflora, and animal prey. Ingestion, however, does not demonstrate nutritional value, and the problem is to know which foods support most insect growth (Cummins 1973). The question is further complicated by species being able to use different foods in different habitats, and by their changing diets with age. Furthermore, mere alterations in the physical properties of the substratum in which they live can cause substantial changes in insect populations (Nilsen and Larimore 1973) and make it difficult to separate substrate preference from food preference. It is worth noting, however, that in general the greatest densities of aquatic insects are recorded from lake outflows, where blackflies and filter-feeding caddisflies collect suspended living material, from wave-washed gravel shores of the littoral zone of rich lakes, from streams receiving domestic sewage, and from sewage filter-beds. It appears, then, that a well-oxygenated, organically rich medium with particles and minute organisms maintained in suspension is most favorable for aquatic insects.

The main strands, and ways of influencing them, may be discussed briefly.

Epilithic algae, especially diatoms, are of importance because they are eaten by the commonest herbivorous or omnivorous insects, e.g. *Baetis*, heptageniids, *Ephemerella*, and chironomid larvae. These frequently comprise the early diet of salmon (e.g. see White 1936). Filamentous algae, e.g. *Cladophora* in moderately hard and slow-moving water, *Oedogonium* and *Bulbochaetae* in faster water, are also of significance. These flourish conspicuously in strong sunlight where they are not dislodged by freshets, e.g. in artificial spawning channels. This dense plant material is apparently not cropped directly by insects to any great extent, but chironomids thrive among their filaments (Whitton 1970), presumably on protozoa, bacteria, and epiphytic algae. The emerging midges and drifting larvae are readily eaten by fry (Mundie 1971a).

Algae respond to increases in light and nutrients — two invariable consequences of man's activities in a watershed — and the overall result is a change in biota from that of cool, dark, stony streams to one characteristic of the lower stretches and stable shallows of large rivers (Hynes 1969). An exploratory study of the effects of additions of inorganic fertilizers to a barren stream demonstrated pronounced local production of algae, with possible benefits to salmon (Huntsman 1948). It is certain that gross production of filamentous forms is not desirable as they obliterate some valuable benthic invertebrates, e.g. Simuliidae (Thut 1969), reduce flows through gravel, and restrict the movements of fish.

Suspended algae can contribute to a major strand. Seston feeders — Simuliidae, Hydropsychidae, the midge *Rheotanytarsus* — eat diatoms, especially in streams flowing from lakes. The record for benthic production in a stream seems to be held by *Cnephia* (Simuliidae); this amounted to 1.48 g/m^2 per day (wet weight) at a lake outflow (Ulfstrand 1968). The example illustrates how benthic production may be locally dominated by a single influence.

The above elementary categories of algae — diatoms on stones, and filamentous and suspended algae — show that, if algal production is to be increased by chemical additives, there must be clear understanding of the food strand which is being strengthened.

Rooted vegetation is indigenous, as a rule, only in lowland waters where velocities are low enough to allow the plants to gain a footing. Vegetation gives cover to fish and provides a substrate for invertebrates which may be sustained via epiphytes. It can be encouraged by habitat manipulation (Tarzwell 1937, Fort and Brayshaw 1961) but only where the annual runoff makes this practicable. The practice seems to be of doubtful economic promise in unproductive waters.

Terrestrial vegetation has, nutritionally, a dual importance; as a source of terrestrial insects to fish, and as a producer of leaf litter entering the stream which, on decomposition, contributes to the major food strand. From both aspects it is probable that deciduous vegetation is the most valuable. Examples of the number of insect species recorded from trees are: oak 284, willow 266, poplar 97, alder 90, fir 16 (Southwood 1961). The range is therefore great. The biomass of insects supported is also, of course, important, as are the kinds of insects. Some species which are common near water, e.g. Empididae, may be rarely eaten by fry. Both the kinds and quantities of insects settling on a stream will vary with distance from the bank (Nelson 1965; Mundie 1969).

A comparison was made in September (J. H. Mundie and D. E. Mounce unpublished data) of aerial insects occurring on the forested and deforested banks of a small coastal stream in B.C. The population in the deforested stretch was 3 times more abundant than that of the treed stretch. Aphids and chironomids were commonest, while Collembola and higher Diptera predominated on the forested banks.

The factors governing the presence of insects on the water are too complex to permit a simple generalization (see Johnson 1969) but certainly sunshine and wind are of consequence. In open

country in temperate regions the mode of abundance of aerial insects is in July or August (Nelson 1965; Evans and Murdoch 1968); at the margins of dark wooded streams, however, a distinct mode may not occur.

It is possible that salmon may be dependent on terrestrial insects in mid- and late summer at times of low flows, and a high occurrence of terrestrial items in the diet may be indicative of a scarcity of aquatic foods (cf. Ellis and Gowing 1957). The biomass of salmon fry in Scottish streams running through immature coniferous forest plantation, with dense shrub vegetation overhanging the banks, was found to be much higher than in streams passing through mature forest (Mills 1969). In other Scottish streams it was noted that the density and biomass of the total 1+ and older salmon and trout might be similar, but where the vegetation provided a rich terrestrial fauna the size for migration was attained more rapidly (Egglishaw 1967). The tree-layer, which largely determines the shrub and ground layers, could, therefore be selectively deforested to promote the greatest abundance of terrestrial foods, and with the warming of streams resulting from removal of trees a significant increase in the numbers of smolts leaving at 1 yr might result.

The dead organic material entering streams from the land consists mainly of leaves and dissolved organic matter. It is probable that deciduous litter is much more valuable to streams than coniferous litter. This important subject has received scant attention, but such evidence as exists suggests that coniferous trees have a deleterious effect on benthos (Huet 1951), although not necessarily on *Gammarus* (Zelinka 1950). The water courses in coniferous forests frequently contain, of course, a mixed vegetation, with willow and alder, for example, occurring in abundance.

In temperate regions, in a mixed deciduous woodland, up to 81% of the fall of tree and shrub litter takes place in the period September to November, and amounts to about 500 g (dry weight)/m^2 per yr (Sykes and Bunce 1970). Wind is the means by which the leaves are transported to streams. The very slow breakdown of this litter is the basis of much invertebrate production. Its rate is affected by temperature and the phosphorus and nitrogen content of the water, and breakdown is accomplished mainly by fungal attack (Kaushik and Hynes 1971). In contrast to the terrestrial situation, disintegration of the leaves is unaffected by the presence or absence of invertebrates (Mathews and Kowalczewski 1969). Nevertheless many benthic species aggregate on the detritus (Egglishaw 1969) and browse on the microflora (Bärlocher and Kendrick 1973). There is, furthermore, an increase in the amount of benthos, per unit weight of detritus, with increasing hardness of the water (Egglishaw 1968).

The role of the dissolved organic matter derived from leaves and from soil is less clear. The potential of this material for supporting invertebrates is seen when mosquito larvae are found thriving in a plastic pail containing only rainwater and some fallen leaves. About 12% of the dry weight of beech leaves is lost in 24 h when they are submerged in water (Egglishaw 1968). The leached materials produce precipitates which become invaded by microorganisms. These in turn are eaten by invertebrates (Lush and Hynes 1973). Leaching is not confined to the autumn, but this is the period when it is at a maximum (see Witkamp 1971).

In considering the relative contribution of decaying leaves and dissolved material it is worth noting that the successive populations of bivoltine species of insects may be numerically similar, i.e. the summer generation may survive as well as the one which passes the winter after the period of leaf-fall. Summer growers are not necessarily at a disadvantage.

The major importance of leaves, therefore, may lie in determining the organic properties of the soil in the catchment area rather than in contributing litter to the stream. If this is so a narrow strip of deciduous riparian vegetation would have little influence on aquatic production.

Can the heterotrophic strand be strengthened? What effect would the addition of organic material to riffles have on benthos? In general, short of pollution, organic enrichment of streams results in an increase in animal production (Hynes 1969), and can benefit salmonids (Ellis and Gowing 1957). A notable example is the addition of sugar (Warren et al. 1964) which produced a sevenfold increase in trout production via *Sphaerotilus* and chironomid larvae. Attention should be given in Canada to the wet decomposition of low quality cereal grain. When wheat grain was added to gravel in a coastal stream in B.C. a twentyfold increase in weight of benthos occurred after 1 mo (Mundie et al. 1973). Most of the biomass was contributed by larvae of the midge *Polypedilum* which would make a very acceptable food for fry. The species is univoltine but it is possible that a multivoltine *Chironomus* could be similarly cultured on decaying grain.

One of the properties of heterotrophic production which makes it difficult to study, but which is an asset for cultural work, is that macroorganisms feed simultaneously on microorganisms dependent on different trophic strands (see Mann 1969).

Availability of Food

Numerous studies (see e.g. Mann 1969) have been directed at the measurement of stock and turnover

of zoobenthos and of fish populations. These sets of measurements can be related, however, only if some measurement of the availability of food exists. Nothing is to be gained from augmenting the stock of invertebrates if the increase cannot be passed on to the fish. Young salmon can feed both off the bottom and on drifting foods (Keenleyside 1962), but in streams, browsing on benthos may be rare and foods may be essentially derived from drift (Kalleberg 1958; Mundie 1971a; Elliott 1973; Metz 1974 for older trout). Selection of foods has been demonstrated (Bryan and Larkin 1972). There is probably some plasticity of habits and they may change with age. What matters, of course, is the reward for a particular feeding action. Because salmonids feed by sight, and moving objects are especially visible, it seems likely that drifting foods are the most readily eaten.

The sources of organic drift (see Waters 1972) in the environmental unit are shown in Fig. 3. Virtually all benthic organisms, irrespective of their feeding habits, and many terrestrial ones, drift, and the numbers moving towards fish are proportional to current velocity and riffle area. The amount of riffle, relative to area of pool, can be modified to some extent by the installation of deflectors and logs (see Shetter et al. 1946; Mundie 1969). Such adjustments to increase drift must be made with regard for cover, which can be limiting at least for brown trout (*Salmo trutta*) (Lewis 1969), Atlantic salmon (McCrimmon 1954), and brook trout (Hunt 1971).

If the most available foods for fry and parr are in the drift then the relation of the diel feeding activities of the fish to diel drift is of interest. Figure 4 shows the relative composition of hourly (P.S.T.) collections of drift in a small coastal creek in B.C. in the third week of May. Items under 160 μ in width are excluded; these were found to be rarely eaten by coho fry. The most abundant drifting items were insect exuviae, and whole edible items made up between 12 and 47% of the total drift. Since salmon fry eat exuviae (Mundie 1971a), half the energy spent on collecting food may be wasted. The edible items comprised 120 species; nymphs of *Baetis* (2 spp.) made up 30% of the total, chironomid larvae (36 spp.) 18%, and terrestrial organisms (19 spp.) 11%. The bulk of the aquatic organisms drift at night; therefore, many items may not be visible to the fish (Tanaka 1970). On this evidence food may be scarce throughout the daylight hours and in excess, but unattainable, at night. The daytime diet consists mainly of exuviae and terrestrial items.

A typical example of salmonid density in spring is 33 alevins/m^2 (Horton et al. 1968). A fry 3.5 cm in length, with a mean number of 12 food items in the fore-stomach might eat, say, 72 invertebrates in 24 h. Across a 1-m width of stream 24,000 food items passed in that time in the example in Fig. 4, i.e. sufficient to feed 333 fry. The benthos in the riffle amounts to 40,000 insects/m^2 (Mundie 1971b). Studies are needed, therefore, to establish how much drift enters fish, how much settles in pools to contribute to drift downstream the following night or to move upstream (see Waters 1972), and how much is lost from the stream. On the evidence that large quantities of drift leave rivers permanently (e.g. Elliott and Corlett 1972) it is clear that much food is not eaten in streams.

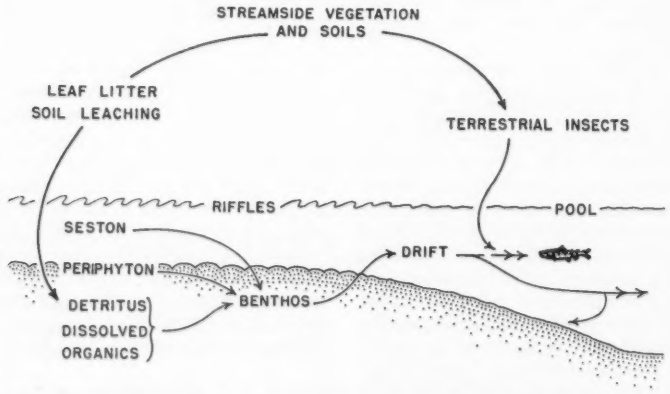

FIG. 3. Invertebrate drift in the environmental unit. Stock of benthos in the riffle amounts to 40,000 insects/m^2; drifting food items amount to 24,000/m width per 24 h; food eaten/fish equals 72 items/24 h.

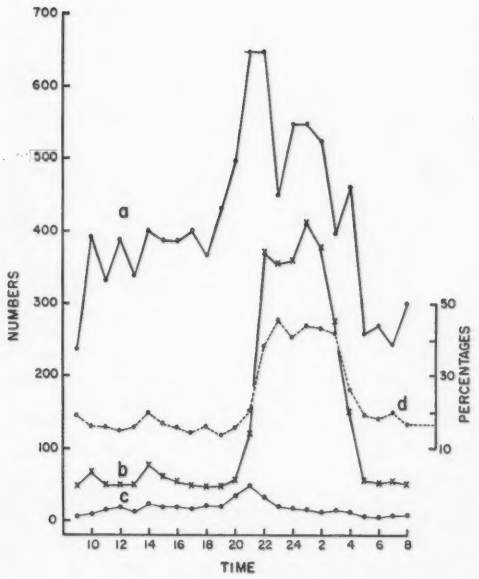

FIG. 4. Diel composition of organic drift in a stream in May. Numbers are means of four samples collected hourly. Mean flow at four stations was 86 cm/s; a, insect exuviae; b, aquatic insects; c, edible terrestrial items; aerial aquatic insects, and emerging insects; d, edible whole items as a percentage of total drift. Scale for d is on the right.

Several ways of making food available to fish are apparent. One is to collect nocturnal drift on screens at intervals down a river and release it to fry in the daytime. Another is to percolate a mixture of air and water through the gravel of riffles so that the benthos is dislodged. Rapid recolonization of the substrate would occur (see Ulfstrand 1968, p. 100). Terrestrial foods can be attracted to the water surface by suspended lights (see Heidinger 1971). On a moorland stream it was noted that only 1% of terrestrial invertebrate production entered the stream surface, but it made up 30% of the food of trout (Rawes and Heal 1973). Greater utilization is therefore possible. Yield of brook trout in an alpine lake was found to be most strongly affected by the quantities of terrestrial insects available to the fish (Walters and Vincent 1973). Natural foods of fry can be supplemented with artificial foods distributed on pools. In streams subject to low flows in summer the combination of small water storage dams at suitable sites in nursery areas (Havey and Davis 1970), with supplementary feeding, might prove profitable.

Implicit in these suggestions is the assumption that the territories of fry and parr are not rigid, and that the level of aggressive behavior of salmon is lowered when the fish are well fed (see Kalleberg 1958; Fenderson and Carpenter 1971; Jenkins 1969). Support for this assumption is provided by the behavior of satiated salmonids in hatcheries.

Discussion

The first point which emerges (apart from the obvious need for further quantification of the variables discussed) is that salmon nursery streams have much more structure, or order, in them than appears at first sight. Features such as meanders or boulders are not fortuitous but essential. A corollary of this is seen in the changes which have taken place in the design of artificial spawning channels. Initially it was thought that control of flow through gravel of selected size-range was required. It is now recognized that freshets, shade, frequent pools, and perhaps a content of sand are necessary; more and more channels are acquiring the appearance of natural streams. Optimized nursery channels would likewise look "natural."

A second point is that the variables which require adjustment must be altered simultaneously; otherwise limitations will persist. Abundant terrestrial food may not compensate for lack of cover, a high ionic content of water for low temperatures, a high benthic production for summer droughts. It is presumably for such reasons that correlations do not appear between ionic content of water and biomass of salmon (Le Cren 1969; Burns 1971). Thirdly, although several ways of manipulating streams to increase salmon production are apparent, the question remains: are they worthwhile? It has been claimed that "the time for intensive management of our trout streams has arrived" (Cooper 1970, p. 153), yet little management is carried out. This is perhaps because of doubt over the cost: benefit ratio, rather than over the soundness of a manipulative procedure.

In terms of costs the most attractive thing to do for salmon is to manage escapement and ensure that good salmon habitat is protected from insidious long-term deterioration as well as obvious immediate damage; habitat protection is the best investment for sustained yield (Power 1973). The application of beneficial measures for increasing smolt production — control of temperature by cutting back vegetation, choice of deciduous riparian trees, supplementary feeding — seems rarely practicable in economic terms. Most streams are inaccessible, they are devoid of trails, and at best have an output of 5000 smolts/1600 m of stream.

When the production of smolts from hatcheries is considered, however, other possibilities present themselves. The success of hatcheries lies in the fact that fish will tolerate high densities if they are well fed. In shallow raceways 100,000 kg of fingerlings can be produced per hectare (cited in Bardach et al. 1972). Their disadvantages lie in high capital and maintenance costs, risks of disease, and loss of genetic heterogeneity in stocks. Insufficient attention, however, seems to have been given to attempting to combine the desirable features of streams with the productive capacity of hatcheries. For much lower costs rearing channels might raise comparable quantities of smolts. At present the Fisheries and Marine Service operates a channel at the Big Qualicum River on Vancouver Island in which a total of half a million chinook, coho, and steelhead smolts are raised on artificial diets in sections of a meandering outdoor channel using 0.4 m^3/s of water. Lights are provided to attract aerial insects. The smolts appear to be of excellent quality and this is attributed to their "natural" setting. Such a rearing facility, however, could contain more stream features. Especially desirable is the riffle–pool sequence so that fish live in a velocity gradient, turbulence aerates the water repeatedly, gravel substrates become colonized with benthos, and drift is generated. Invertebrates can act as scavengers on the waste foodstuffs and feces.

It is the combination of metabolic properties and transporting properties — unique in ecosystems — which make streams suitable for aquaculture of salmonids. The constant influx of oxygen in high-gradient streams implies that substantial additions of organic materials can be made to the substratum, and their conversion to cellular materials can still be carried out aerobically. This cannot be done in standing-water systems which are dependent on wind-induced turbulence for oxygen influx. The maintenance of aerobic conditions is a basic requirement of the salmonid habitat; and the energy yield from aerobic oxidation is likely to be about 5 times greater than that from fermentation (see Brock 1966). The potential for increasing invertebrate stock is therefore promising; so also is the opportunity for making it available to fish. Recolonization from upstream is continuous.

Rearing channels are not without precedent (see Bregnballe 1963 for a description of a low-velocity trout channel) nor without difficulties. There are problems associated with river intakes, but they are familiar to engineers (Popkin and Charbonneau 1973). Choice of site is restricted by available head. Serious loss of stock from predators (Elson 1962; Onodera 1962), long-term accumulation of fines owing to the absence of freshets, and encroachment of bank and aquatic vegetation for the same reason,

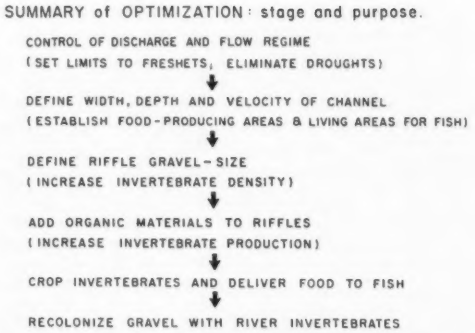
SUMMARY of OPTIMIZATION: stage and purpose.
CONTROL OF DISCHARGE AND FLOW REGIME
(SET LIMITS TO FRESHETS; ELIMINATE DROUGHTS)
DEFINE WIDTH, DEPTH AND VELOCITY OF CHANNEL
(ESTABLISH FOOD-PRODUCING AREAS & LIVING AREAS FOR FISH)
DEFINE RIFFLE GRAVEL-SIZE
(INCREASE INVERTEBRATE DENSITY)
ADD ORGANIC MATERIALS TO RIFFLES
(INCREASE INVERTEBRATE PRODUCTION)
CROP INVERTEBRATES AND DELIVER FOOD TO FISH
RECOLONIZE GRAVEL WITH RIVER INVERTEBRATES

FIG. 5. Stages in optimizing stretches of a river for raising salmon. Definition of gravel size includes boulders, cobbles, and gravel for summer and winter cover. Invertebrates are cropped to be released to fish; see text.

are likely to present problems, but they are probably surmountable because of the small scale of the operation. Winter cover, which is limiting for chinook and trout (Bjornn 1971) can be provided.

What proportions of natural foods could be maintained in the diet, and what densities of fish could be raised most economically, are questions for experimentation. The application of the principles (summarized in Fig. 5) calls for side-channels to be made alongside rivers which can supply adequate flows at minimum stage. If it proved economically feasible to harness sections of rivers in this way, with the river supplying the stock of fry and doing most of the work, then the salmon resource could be kept geographically dispersed, and rivers, being indispensable for the operation, would receive protection from abuse.

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Fish Farming International, No, 1, 2. *Edited by Peter Hjul. Fishing News (Books) Ltd., Surrey, England, 1973, 1974, each issue about 150 p., £3.00.*

This journal largely succeeds in its attempt to provide a worldwide review of current developments in fish farming as a food-producing industry. The first number of Fish Farming International appeared in 1973. Number 2 came in the spring of 1974 with an announcement of a third number in autumn 1974 and the intention to begin quarterly publication in spring 1975.

The articles in No. 1 and 2 include submitted papers, conference papers, product news, advertisements, progress reports, plans for fish farming ventures, advice to potential aquaculturists, reports from aquaculture-related meetings, and editorial comment on fish farming. These represent government, industry, feed, chemical and manufactured product suppliers, and the small fish farm operator. It is perhaps not surprising that the publishers of Fishing News, Fishing News International and a number of leading books on aquaculture have achieved such good coverage of world aquacultural activities. There was some — but not unnecessary — repetition between No. 1 and 2.

The editorial policy is a refreshing one. It is not suggested that fish farming will take the place of traditional capture of natural stocks by "hunting." Instead, the editors see fish farming as a potentially great industry in its early developmental stages which together with traditional fish "hunting" must grow in production to meet the world's increasing demands for quality protein. They do not agree with the "small-prospect" attitude towards aquaculture of many fisheries scientists, industrialists, and even some fish farmers who see aquaculture as subordinate to taking fish by capture. They urge fish farmers to consider not only the high value species such as salmonids, shrimp, flatfish, etc., which are raised for gourmet tastes for the thousands, but also fish as a protein to feed the millions. With this editorial background, No. 1 and 2 of Fish Farming International provide a useful collection of material to all concerned with raising fish for food.

The format of the journal leaves something to be desired. It is not always clear who has authored articles or whether they are produced by the editorial staff. Various sized headline print and sometimes horizontal lines are used to separate articles. Standardization of type size used for article titles, subheadings, etc., would make for easier article selection and skimming. A complete listing of articles in the table of contents would be helpful.

Advertisements for products used in fish farming are a useful part of the journal. Some of them are clearly set out in blocks and easily recognized as such. Advertisements for the various books produced by Fishing News (Books) Ltd. seem to be overstressed by repeated coverage in block advertisements, reference in articles, and editorial comments.

In an article (F. F. I. No. 1, p. 137–138) on the use of a popular anesthetic for fishes it is stated that the chemical is harmless to fish being treated and to people handling the fish and the drug. This statement needs some qualification. Anesthetics are potentially dangerous to fish and to people handling them. An advertisement for the same product in F.F.I. No. 2 stresses the usefulness of the anesthetic without making unwarranted statements about its harmlessness.

In some instances where this reviewer is familiar with activities reported on in F.F.I., significant features have been missed. In two accounts of Norway's salmon farming test station at Sunndalsöra (F.F.I. No. 1, p. 34–35; F.F.I. No. 2, p. 23–24), no mention is made of the elevated temperature of the power station effluent which permits year-round fish production at near optimum temperature and the production of salmon smolts in a much shorter time than in most hatcheries and in nature.

Fish Farming International deals with more than fishes. There are a number of articles on molluscs and crustaceans. It would seem logical to include culture of aquatic plants as well. Polyculture might embrace both plants and animals, not just fin fishes. A new title encompassing fishes, invertebrates, and plants might be appropriate.

The overall usefulness of F.F.I. is high so far. Hopefully, this will continue with the production of No. 3 and the intended quarterly publication beginning in 1975. An increased content of submitted scientific and popular articles is invited. This will increase the usefulness of the journal. A continued enlightened editorial policy and broad coverage of the fish farming field will establish F.F.I. as indispensable to all associated with aquaculture.

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Recent Studies on Investigations Published in Other Periodicals

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating establishment.

Récentes études sur des recherches mentionnées dans d'autres périodiques

Jusqu'en 1970, ces articles étaient réimprimés, puis reliés dans un volume annuel d'étude, comprenant deux parties. Les études de 1969, partie 2, incluant le n° 1355, ont été les dernières à être reliées. Nous n'en faisons désormais plus la reliure ni la distribution. Nous continuons cependant de numéroter les articles et d'en établir des listes. On peut donc se les procurer, sous leur forme réimprimée seulement, de l'auteur ou du service dépositaire.

1977. PARSONS, L. S. 1973. Meristic characteristics of Atlantic herring, *Clupea harengus harengus* L., stocks in Newfoundland and adjacent waters. Int. Comm. Northwest Atl. Fish. Res. Bull. 10: 37–52. (9)

1978. PITT, T. K. 1973. Assessment of American plaice stocks on the Grand Bank, ICNAF divisions 3L and 3N. Int. Comm. Northwest Atl. Fish. Res. Bull. 10: 63–77. (9)

1979. TEMPLEMAN, W. 1973. Distribution and abundance of the Greenland halibut, *Reinhardtius hippoglossoides* (Walbaum), in the Northwest Atlantic. Int. Comm. Northwest Atl. Fish. Res. Bull. 10: 83–98. (9)

1980. SCOTT, J. S. 1973. Otolith structure and growth in northern sand lance, *Ammodytes dubius*, from the Scotian Shelf. Int. Comm. Northwest Atl. Fish. Res. Bull. 10: 107–115. (6)

1981. CADDY, J. F., AND T. D. ILES. 1973. Underwater observations on herring spawning grounds on Georges Bank. Int. Comm. Northwest Atl. Fish. Res. Bull. 10: 131–139. (6)

1982. MAY, A. W., AND W. H. LEAR. 1973. Gutted weight versus total length of Atlantic salmon at West Greenland. Int. Comm. Northwest Atl. Fish. Res. Bull. 10: 163–166. (9)

1983. MILLER, R. J. 1974. Distribution and biomass of an estuarine ctenophore population, *Mnemiopis leidyi* (A. Agassiz). Chesapeake Sci. 15: 1–8. (9)

1984. RANTALA, R. T. T., AND D. H. LORING. 1973. New low-cost teflon decomposition vessel. At. Absorpt. Newsl. 12: 97–99. (7)

1985. BISHOP, C. A., AND W. THRELFALL. 1974. Helminth parasites of the common eider duck, *Somateria mollissima* (L.), in Newfoundland and Labrador. Proc. Helminthol. Soc. Wash. 41: 25–35. (9)

1986. BUTTKUS, H. 1974. On the nature of the chemical and physical bonds which contribute to some structural properties of protein foods: A hypothesis. J. Food Sci. 39: 484–489. (2)

1987. ZINCK, M. E., AND R. F. ADDISON. 1974. The fate of 2-, 3-, and 4- chlorobiphenyl following intravenous administration to the thorny skate (*Raja radiata*) and the winter skate (*Raja ocellata*). Bull. Environ. Contam. Toxicol. 2: 52–61. (7)

1988. BERNARD, F. R. 1974. The genus Calyptogena in British Columbia with a description of a new species (Bivalvia, Vesicomyidae). Venus Jap. J. Malacol. 33: 11–22. (1)

Interpretive Articles Series

This new series commenced in 1966. The articles interpret work already published in primary journals. They appear at irregular intervals in various technical publications. Frequently an article essentially duplicates, condenses, or is based on an article in another series.

Articles explicatifs

Cette série a débuté en 1966. Les articles donnent une explication de travaux déjà publiés dans des revues prioritaires. Ils paraissent à intervalles irréguliers, dans diverses publications techniques. Souvent, un article se basera sur un écrit d'une autre série, le condensera ou le reproduira presque tel quel.

316. PRITCHARD, G. I. 1974. Fish farming and its role in nutrient recycling. Symposium on "Recycling of food processing industrial, and farm wastes in animal feeding," sponsored by the Canadian Society of Animal Science, Charlottetown, P.E.I., June, 1972. 23 p. (10)

317. WALDICHUK, M. 1973. Conference report. IMCO International Conference on Marine Pollution, London, Oct. 8–Nov. 2, 1973. Ocean Manage. 1(1973): 277–286. (3)

Manuscript Report Series

This series includes unpublished preliminary reports and data records not intended for general distribution. They should not be referred to in publications without clearance from the issuing establishment and without clear indication of their manuscript status.

Rapports manuscrits

La série comprend des articles d'information et des rapports préliminaires non publiés, qui ne sont pas destinés à la distribution générale. Ils ne doivent pas être donnés en références sans l'assentiment du service dépositaire et sans une indication claire de leur forme manuscrite.

WILDER, D. G. 1974. Inshore and offshore lobster stocks. Fish. Res. Board Can. MS Rep. 1293: 14 p. (6)

GODFREY, H., AND E. A. R. BALL. 1974. Data record, with comments fall and winter fishing for juvenile salmon in Georgia Strait 1972–73. Fish. Res. Board Can. MS Rep. 1294: 59 p. (1)

WILDISH, D. J., W. V. CARSON, A. J. WILSON, AND J. H. HULL. 1974. Effects of a neutral-sulphite, pulp effluent on some chemical and biological parameters in the l'Etang Inlet, New Brunswick. L'Etang Inlet Survey II. Fish. Res. Board Can. MS Rep. 1295: 11 p. (6)

MCDONALD, J., AND R. A. BAMS. 1974. Research needs in salmon enhancement. Fish. Res. Board Can. MS Rep. 1296: 9 p. (1)

QUAYLE, D. B. 1974. Variance in marine borer attack in connection with a treated wooden pile experiment at Crofton, B.C. Fish. Res. Board Can. MS Rep. 1297: 15 p. (1)

CHILTON, D. E. 1974. An inventory of biological samples taken in the commercial groundfish fishery in British Columbia in 1973 (data record). Fish. Res. Board Can. MS Rep. 1298: 21 p. (1)

CHILTON, D., D. LOVELY, AND C. R. FORRESTER. 1974. IDB-supported *Gail Bernice* groundfish cruises 73–10 to 73–14 to Hecate Strait and Queen Charlotte Sound, August 3–December 16, 1973 (data record). Fish. Res. Board Can. MS Rep. 1299: 59 p. (1)

GLOVA, G. J. AND J. C. MASON. 1974. Interactive ecology of juvenile salmon and trout in streams. I. Program during 1973. Fish. Res. Board Can. MS Rep. 1300: 36 p. (1)

WESTRHEIM, S. J., W. R. HARLING, D. DAVENPORT, AND M. S. SMITH. 1974. *G. B. Reed* groundfish cruise No. 74–1, February 5–22, 1974 (data record). Fish. Res. Board Can. MS Rep. 1301: 15 p. (1)

JONES, B. C. 1974. *G. B. Reed* groundfish cruise No. 74–2, March 4–15, 1974 (data record). Fish Res. Board Can. MS Rep. 1302: 15 p. (1)

NARVER, D. W., AND B. C. ANDERSEN. 1974. Fish populations of Carnation Creek and other Barkley Sound streams — 1970–1973: data record and program report. Fish. Res. Board Can. MS Rep. 1303: 115 p. (1)

FOULKES, T. J., S. M. POLAR, J. M. BABINEAU, AND P. J. G. CARROTHERS. 1974. Instruction manual modified EG & G, 200 A/ 210 A, automatic, underwater, photographic system. Fish. Res. Board Can. MS Rep. 1304: 36 p. (6)

MARKLE, G. 1974. On the distribution and development of larval swordfish (*Xiphias gladius* L.) in the western Atlantic. Fish. Res. Board Can. MS Rep. 1305: 4 p. (6)

WILDISH, D. J. 1974. Total body lipid and brain acetylcholinesterase activity of brook trout exposed to finitrothion. Fish. Res. Board Can. MS Rep. 1306: 15 p. (6)

WESTRHEIM, S. J. 1974. Echo-sounder and trawl survey of Queen Charlotte sound and southern Hecate Strait, 1972–73. Fish. Res. Board Can. MS Rep. 1307: 43 p. (1)

BILTON, H. T., D. W. JENKINSON, AND R. M. HUMPHREYS. 1974. Relationship between vertebra diameter and fish length and usefulness of making vertebra of young coho salmon (*Oncorhynchus kisutch*) with oxytetracycline. Fish. Res. Board Can. MS Rep. 1308: 7 p. (1)

PETERSON, R. H. 1974. Lethal response of brook trout (*Salvelinus fontinalis* (Mitch.)) to dissolved copper as affected by prior sublethal exposure to the metal. Fish. Res. Board Can. MS Rep. 1309: 9 p. (6)

CHILTON, D., AND D. LOVELY. 1974. Biological observations made during IDB-supported cruises of the *Gail Bernice* March 26, 1973–March 21, 1974 (data record). Fish. Res. Board Can. MS Rep. 1310: 15 p. (1)

Technical Report Series

Technical Reports are research documents that are of sufficient importance to be preserved, but which for some reason are not appropriate for primary scientific publication. Inquiries concerning any particular Report should be directed to the issuing establishment.

Rapports techniques

Les rapports techniques sont des documents de recherche qui revêtent une assez grande importance pour être conservés mais qui, pour une raison ou pour une autre, ne conviennent pas à une publication scientifique prioritaire. Pour toute demande de renseignements concernant un rapport particulier, il faut s'adresser au service responsable.

446. WHITAKER, J., AND J. MARTIN. 1974. The cage rearing of rainbow trout in Precambrian lakes. Fish. Res. Board Can. Tech. Rep. 446: 13 p. (4)

447. MACFARLANE, S. A., AND M. YAMAMOTO. 1974. The squid of British Columbia as a potential fishery resource — a preliminary report. Fish. Res. Board Can. Tech. Rep. 447: 36 p. (2)

448. GORDON, D. C. JR., AND P. D. KEIZER. 1974. Hydrocarbon concentrations detected by fluorescence spectroscopy in seawater over the continental shelf of Atlantic Canada — background levels and possible effects of oil exploration activity. Fish. Res. Board Can. Tech. Rep. 448: 24 p. (7)

449. SHRIVASTAVA, H. 1974. Macrobenthos of Lake Huron. Fish. Res. Board Can. Tech. Rep. 449: 45 p. (11)

450. WILDISH, D. J., AND R. L. PHILLIPS. 1974. An identification strategem for benthos collected to assess marine and estuarine pollution. Fish. Res. Board Can. Tech. Rep. 450: 31 p. (6)

451. WESTRHEIM, S. J., AND N. S. FADEEV. 1974. Echo-sounder and trawl net calibration studies with the *G. B. Reed* and *Ogon* September 1972. Fish. Res. Board Can. Tech. Rep. 451: 23 p. (1)

452. KENNEDY, W. A. 1974. Sablefish culture — final report. Fish. Res. Board Can. Tech. Rep. 452: 15 p. (1)

Translation Series

The following translations have been prepared by or for the Fisheries and Marine Service, for use in its investigations. A limited number of copies are available for distribution as long as the supply lasts, or may be obtained by interlibrary loan, from the station where the translation has been deposited. Information on translations generally may be obtained from the Office of the Editor.

Traductions

Les traductions dont il est question ci-après ont été préparées pour ou par le Service des pêches et des sciences de la mer, à des fins de recherche. On peut en obtenir un nombre limité d'exemplaires ou se les procurer par prêts entre bibliothèques, aux stations dépositaires de ces textes. On peut en général se renseigner sur ces traductions au Bureau du rédacteur.

WIBORG, K. F., AND B. BÖHLE. 1968. Occurrences of edible shellfish (bivalves) in Norwegian coastal waters (with a section on marine gastropods). Fisk. Gang 54(9): 149–161. (Transl. from Norwegian by Fish. Res. Board Can. Transl. Ser. No. 2978, 1974) (9)

PURAZRANG, H., AND J. WURZIGER. 1973. Investigation and evaluation of deep-fried foods. Fette — Seifen — Anstrichm. 75: 260–265. (Transl. from German by Fish. Res. Board Can. Transl. Ser. No. 2979, 1974) (8)

KOSSWIG, K. 1973. Additional notes on the method of age determination in redfish (*Sebastes marinus* L. and *S. mentella* Travin). Ber. Dtsch. Wiss. Komm. Meeresforsch. 23: 84–89. (Transl. from German by Fish. Res. Board Can. Transl. Ser. No. 2980, 1974) (9)

FLO, A., N. HAGEN, AND V. MOHR. 1972. Fat tissues in fish. Report on preliminary studies during the period 1.9–31.12.1972. Forskningsprosjeckt B.0103. 3858 Nor. Tek.-Naturvitensk. Forskningsråd p. 1–16. (Transl. from Norwegian by Fish. Res. Board Can. Transl. Ser. No. 2981, 1974) (8)

YAMAMOTO, Y. 1972. Fatty acid composition of blue-green algae. Seibutsu Kagaku 24(3): 130–136. (Transl. from Japanese by Fish. Res. Board Can. Transl. Ser. No. 2982, 1974) (8)

VINBERG, G. G. 1961. Modern conditions and problems in the study of the primary production of waters. Pervinchnaya Prod. Morei Unutr. Vod p. 11–24. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2983, 1974) (11)

The Fourteenth Supplement to List of Articles in the Translation Series No. 2301–2825, with Indexes to Authors and Translators, is now available from the Department of the Environment, Fisheries and Marine Service, Office of the Editor, 116 Lisgar St., Ottawa, Ont. K1A 0H3.

Le quatorzième supplément de la liste des articles dans la série des traductions n° 2301–2825, avec index d'auteurs et de traducteurs, est maintenant disponible au Ministère de l'Environnement, Service des pêches et des sciences de la mer, Bureau du directeur de la publication, 116 rue Lisgar, Ottawa, Ontario K1A 0H3.

ADDITIONAL PUBLICATIONS/ PUBLICATIONS ADDITIONNELLES

The Service also publishes a series of *Bulletins*, each on a specific topic. These are for sale by Information Canada, Ottawa, and remittances must be in advance, payable in Canadian funds fo the order of the Receiver General of Canada. *Bulletins* are also available from local booksellers and Information Canada bookshops throughout Canada.

The *Annual Report* of the Board and the biennial *Review* of investigations are available free, on request. All publications may be referred to at establishments located at: Ottawa, Ont.; Nanaimo, Vancouver, and West Vancouver, B.C.; Winnipeg, Man.; Ste. Anne de Bellevue, Que.; St. Andrews, N.B.; Halifax and Dartmouth, N.S.; and St. John's, Nfld.

Le Service publie également une série de *Bulletins*, chacun traitant sur un sujet spécifique. Les publications de l'ORP sont vendues par Information Canada à Ottawa. Les remises, payables d'avance en monnaie canadienne, doivent être établies à l'ordre du Receveur général du Canada. Les *Bulletins* sont aussi en vente chez votre libraire et dans les librairies d'Information Canada.

Vous pouvez obtenir le Rapport Annuel et la Revue biennale gratuitement. On peut consulter ces ouvrages aux établissements situés à Ottawa (Ont.) à Nanaïmo, à Vancouver, et à Vancouver Ouest (C.-B.), à Winnipeg (Man.), à Ste-Anne-de-Bellevue (P.Q.), à St-Andrews (N.-B.), à Halifax et à Dartmouth (N.-E.), et à St-Jean (T.-N.).

Journal
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Guide to Authors

A more detailed guide on preparation of manuscripts from theses is available from the Office of the Editor.

Content

We invite submission of manuscripts in the broad field of fisheries management, ocean science, and aquatic environments relevant to Canada.

***Articles*:** Reports of research studies of broad scope that are original contributions to science.

***Notes*:** Differ from papers on the basis of scope or completeness, rather than quality of research. They may be on problems with narrow, readily defined limits; on work that is largely confirmatory; on methodology or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued; or on preliminary findings that warrant publication before problems are resolved. They may be organized with as much flexibility as articles except that they have no centered headings and should occupy no more than four printed pages.

***Reviews*:** Detailed articles that are critical appraisals of broad areas of investigation, particularly in fields that have shown rapid development in recent years.

***Perspectives*:** Essays of opinion or hypothesis on fisheries, ocean science, aquatic environments, or related topics that are of concern to the professional and layman. References are desirable; informal style is welcomed.

***Letters*:** Opinion on topics recently under discussion in the *Journal*; preferred maximum length, 500 words. The Editor reserves the right to shorten letters and to make other changes to accord with the style of the *Journal*.

Editorial Practices

The *Style Manual for Biological Journals* (3rd ed. American Institute of Biological Sciences, 3900 Wisconsin Ave. NW., Washington, D.C.) is used as the authority in matters of form. The standards for spelling are: *Webster's Third New International Dictionary* and *le Grand Larousse Encyclopedique*. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. The *Journal* follows the names and spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (Third Edition, 1970) Special Publication No. 6 of the American Fisheries Society.

Series titles should be avoided. An *abstract* is required for all manuscripts, and should state concisely in less than 250 words, what was done, the essentials of the new knowledge, and the main conclusions. Abstracts are published in English and French; those submitted in one language only are translated by the *Journal*.

The Editor normally submits each manuscript to at least two *referees*. Contributors are encouraged to suggest at least four names to be added to the list from which referees may be selected.

Illustrations should be submitted in triplicate as photographs (*glossy prints*) no larger than a standard page and must accompany the manuscript. Prints should not be mounted, stapled, or clipped. Symbols, letters and numerals must be neat, and at least 1.5 mm high when the illustration is reduced to the appropriate size. Drawings should, when possible, be grouped to facilitate comparisons.

Proofs are sent to the author, who is responsible for correcting only errors in typesetting. *Alterations to the content must be avoided.*

Fifty *reprints*, without covers, are supplied free. Additional copies may be ordered when the galley proof is returned. A charge of $30.00 per page is made for articles published. When submitting a manuscript, an author should state that his institution will accept this charge.

Checklist on Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables or figures and the text, or within the text? Should some of the data be made available separately, in a manuscript report or at a data repository? If you use statistical analysis are the statistics subordinate to the biology?

Would any of the text be clearer if condensed? Are summary statements given at the beginnings of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data?

Are generalizations on the *results* adequate? Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

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Guide des Auteurs

On peut se procurer au bureau du Directeur de la publication un guide plus détaillé pour la préparation de manuscrits à partir de thèses.

Sujets

Nous désirons recevoir des manuscrits s'insérant dans le vaste domaine de la gestion des pêches, des sciences de la mer et des milieux aquatiques au Canada.

***Articles*:** Comptes rendus d'études d'une portée générale, constituant des contributions inédites à la science.

***Notes*:** Se distinguent des articles par la portée et le degré d'achèvement plutôt que par la qualité des recherches. Elles peuvent traiter de problèmes particuliers et bien définis; de travaux qui sont en grande partie confirmatoires; de méthodologie ou de techniques de recherche; de progrès de la connaissance découlant d'études plus vastes; de développements dans des projets abandonnés; ou de découvertes préliminaires qui méritent d'être publiées avant la solution de problèmes à l'étude. Les notes peuvent être présentées d'une façon aussi souple que les articles, mais elles ne doivent pas porter d'en-têtes centrés ni occuper plus de quatre pages.

***Critiques*:** Articles détaillés constituant une appréciation critique de vastes champs de recherches, particulièrement dans les domaines ayant connu de rapides progrès au cours des dernières années.

***Perspectives*:** Essais de l'opinion ou de l'hypothèses sur les pêches, les sciences de la mer, les milieux aquatiques, ou sur des sujets connexes qui intéressent professionnels et profanes. Il est préférable de donner des références et d'écrire, si possible, dans un style simple.

***Lettres*:** Opinions sur des sujets exposés dans le *Journal*. Longueur maximale préférable: 500 mots. Le directeur se réserve le droit de raccourcir les lettres et de faire d'autres modifications dans le style du *Journal*.

Dispositions générales

Le *Style Manual for Biological Journals* (3e éd., publié par l'American Institute of Biological Sciences, 3900 avenue Wisconsin N.O., Washington, D.C.) sert de guide quant à la forme de présentation. Le *Webster's Third New International Dictionary* et le *Grand Larousse Encyclopédique* font autorité pour l'orthographe des mots. Les titres de périodiques sont abrégés suivant les *Biological Abstracts*. Les références bibliographiques dans le texte doivent suivre le système nom de l'auteur-année. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No. 6 de l'American Fisheries Society.

Il faut éviter les *articles en série*. Un *résumé* doit accompagner chaque texte et doit exposer brièvement (moins de 250 mots) le travail accompli, les points essentiels des connaissances acquises et les principales conclusions. Les résumés paraissent en anglais et en français, le *Journal* veillant à la traduction dans le cas des présentations unilingues.

Le directeur soumet d'ordinaire chaque texte à l'approbation de deux *arbitres* ou plus. Nous encourageons les collaborateurs à suggérer au moins quatre personnes dont les noms pourraient figurer sur la liste des arbitres.

Les *illustrations* doivent être sous forme de photos (*sur papier glacé*), ne pas dépasser les dimensions d'une page normale et accompagner le texte. Elles ne doivent pas être montées, coupées ni brochées. Les symboles, lettres ou chiffres doivent se détacher avec précision et avoir au moins 1,5 mm de hauteur lorsque l'illustration est réduite à la grandeur voulue. Les dessins doivent, autant que possible, être groupés pour faciliter l'étude comparative.

L'auteur doit revoir les *épreuves* qu'on lui remet et en corriger seulement les erreurs typographiques. *Il doit éviter de changer le contenu du texte.*

Cinquante *tirés à part*, sans couverture, sont fournis gratuitement. L'auteur peut profiter du renvoi des épreuves en placards pour commander des tirés à part supplémentaires. Les frais sont de $30.00 par page pour la publication des articles. Quand un auteur soumet un manuscrit, il doit spécifier que son institution accepte les frais.

Listes de contrôles pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les éditeurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes au but de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou dans le texte même? Est-ce que certaines données devraient être présentées séparément, dans un rapport manuscrit ou dans un dépôt de données? Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la biologie?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il concis, tout en étant représentatif du contenu du rapport?

Le *résumé* donne-t-il l'essentiel des connaissances nouvelles?

L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème?

La section *matériel et méthode* contient-elle le minimum de détails qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données?

Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions?

La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes?

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Guide for Typists Preparing Copy

Read "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 21.5 × 28 cm (8.5 × 11 inches). If possible, use paper with the lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numerals. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (see Appendix A). Type the abstract on a separate page (see Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send the ribbon copy and two other clear copies to the Office of the Editor.

Tables: Examine the current volume of the *Journal* to see how tables are set up (see Appendix C).
Note especially the following concerning *numbered tables:*

Type each on a separate page, using paper without the lines numbered.

Number them with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines (see Appendix C).

Illustrations: Send at least 3 *glossy prints* (no larger than 21.5 × 28 cm) of each illustration with the manuscript. Type the author's name and figure number on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical Expressions, Equations, and Formulas: Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (see Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter *l* and the numeral *one*, and between the capital letter $\overline{O}$ and the numeral *zero*. Make other explanatory notes similarly.

References: Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style used in the *Journal* (see Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list; if an abbreviation is in doubt, type the name in full.

Spellings: About 100 words common in fisheries journals are listed in Appendix F.

Appendices: Appendices A to F are available on request from the Office of the Editor.

Journal Fisheries Research Board Canada

Guide pour les secrétaires d'auteurs

Lisez le "Guide des Auteurs" reproduit dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de dactylographier le texte ou d'en agencer les détails, suivez la pratique du *Journal*.

Dactylographiez le manuscrit sur papier blanc de bonne qualité, et non sur papier pelure, mesurant de préférence 21.5 × 28 cm (8.5 × 11 pouces), laissant toujours une marge d'au moins 2.5 cm. Si possible, employez un papier avec lignes numérotées en marge.

Tout le manuscrit, y compris tableaux, légendes de figures, renvois et références bibliographiques, devra être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique. Mettez en retrait la première ligne de chaque paragraphe du texte et de chaque renvoi.

Dans les titres et les en-têtes, n'employez la majuscule que pour le premier mot et les noms propres. Pour les petites majuscules, employez les minuscules du dactylographe.

Indiquez tous les renvois du texte par des chiffres arabes suscrits. Numérotez les renvois consécutivement par tout le texte, à commencer par ceux de la page titre. Placez chaque renvoi directement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit contenir que le titre de la publication, le nom et l'affiliation de ou des auteurs, les renvois appropriés et le titre courant (voir Appendice A). Dactylographiez le résumé sur une page séparée (voir Appendice B). Numérotez chaque page, à commencer par la page titre, et y compris celles qui contiennent les tableaux et la légende des figures.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie et ainsi que deux copies lisibles au bureau du Directeur de la publication.

Tableaux. Consultez une copie du *Journal* pour la composition des tableaux (voir Appendice C).

Portez une attention particulière à ce qui suit au sujet des *tableaux numérotés*.

Dactylographiez chaque tableau sur feuille séparée, vous servant de papier sans lignes numérotées.

Numérotez les tableaux en chiffres arabes.

Tracez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, rarement ailleurs; ne tracez jamais de lignes verticales; laissez plutôt des espaces supplémentaires.

Indiquez les renvois dans les tableaux par les minuscules suscrites et placez les renvois au bas du tableau.

Placez les tableaux à la suite des références bibliographiques.

A l'endroit où le tableau est mentionné pour la première fois, indiquez dans la marge "Tableau — près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, sont dactylographiés avec le texte et ne requièrent que rarement des lignes horizontales (voir Appendice C).

Figures: Envoyez avec le manuscrit trois photos *sur papier glacé* (21,5 × 28 cm ou moins) pour chaque illustration. Dactylographiez le nom de l'auteur et le numéro de la figure sur une lisière de papier que vous collerez au dos. N'écrivez pas à la main ou à la machine sur la photographie même. En plus, dactylographiez les légendes, dans l'ordre, sur feuille séparée, intitulée "Légendes des figures." Intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge "Fig. — près d'ici."

Equations, formules et expressions mathématiques: Laissez trois espaces au-dessus et au-dessous des lignes renfermant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous d'équations et de formules. Placez chaque composante des différentes expressions dans la position qui lui est propre (voir Appendice D).

Indiquez clairement tous les symboles. Epelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre *l* et le chiffre *un*, et entre la majuscule *O* et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Références: Vérifiez le texte afin de vous assurer que toutes les références apparaissant dans la liste bibliographique sont bien mentionnées dans le texte. Dactylographiez à double interligne dans le style en usage dans le Journal (voir Appendice E). Les noms de revues sont abrégés suivant les *Biological Abstracts*; en cas de doute, écrivez le nom au long.

Orthographe: Une liste d'environ 100 mots communément employés dans les périodiques sur les pêches est donnée en Appendice F.

Appendices: On peut se procurer une copie des Appendices A à F sur demande au bureau du Directeur de la publication.

BULLETIN 189

Treatment of Fish Processing Plant Wastewater

F. G. CLAGGETT/J. WONG

During the processing of seafood, from 0.5 to 10 gal of water per lb of fish processed may be used for conveying, processing, and plant cleanup. The resultant effluent contains from 2 to 30 lb of organic solids per 1000 lb of fish processed.

Despite the variety of approaches that regulatory agencies are taking in selecting standards, fish processing plants can expect to be required to supply some degree of treatment to their effluents. A review is included of some waste treatment methods which might be expected to apply in the fish processing industry.

As most of the methods mentioned are either poorly suited to the industry or are expensive, we have studied the application of fine screening and dissolved air flotation. Data obtained from operation of a demonstration treatment plant indicates reduction of 25–40% in biochemical oxygen demand (BOD) and suspended solids can be achieved by fine screening, and a further 75% BOD and 95% suspended solids removed by chemical treatment and dissolved air flotation.

These studies have also shown that if the solids recovered by the screens are marketed as fish meal, the value received will, in most cases, offset of the cost of the screens. Solids for fish meal production can also be recovered from the sludge produced by air flotation, but the recovery is more difficult and expensive, and hence the value received will do little more than offset the operating costs of the treatment.

Lors du traitement des produits marins, des volumes d'eau variant entre 0.5 et 10 gal d'eau par lb de poisson transformé peuvent être employés pour le système transformeur, la transformation du poisson et le nettoyage de l'usine. L'effluent qui en résulte contient de 2 à 30 lb de solides organiques par 1000 lb de poisson traité.

En dépit de la variété des méthodes d'attaque adoptées par les organismes de réglementation dans le choix des normes, les usines de transformation du poisson peuvent s'attendre à devoir traiter leurs effluents jusqu'à un certain point. Le présent Bulletin passe en revue quelques méthodes de traitement susceptibles d'application dans l'industrie de la transformation du poisson.

Comme la plupart des méthodes mentionnées conviennent mal à l'industrie ou sont dispendieuses, nous avons étudié l'application du tamisage fin et de la flottaison à l'air dissous. Les données résultant de l'opération d'une usine de démonstration indiquent que le tamisage fin peut produire une réduction de 25 à 40% de la demande biochimique en oxygène (DBO) et des solides en suspension. Le traitement chimique et la flottaison à l'air dissous peuvent entraîner une réduction additionnelle de 75% de la DBO et l'enlèvement de 95% des solides en suspension.

Ces études démontrent de plus que, si les solides récupérés sur les tamis sont mis en marché sous forme de farine de poisson, la valeur reçue compensera, dans la plupart des cas, le coût des tamis. Les solides servant à la production de farine de poisson peuvent également être récupérés de la boue produite par flottaison à l'air, mais la récupération en est plus difficile et plus dispendieuse, de sorte que la valeur reçue ne fera pas beaucoup plus que couvrir les coûts d'opération du traitement.

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